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CALCIUM AND PHOSPHORUS STUDIES

XII. Six Years' Clinical Experience with Viosterol in the Prevention and Treatment of Rickets, Tetany, and Allied Diseases

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Submitted for publication September 20, 1935

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PART I

1. INTRODUCTION

The introduction of viosterol as an antirachitic agent brought with it many problems of clinical importance. (1) Is viosterol as efficacious as cod liver oil in the prevention and cure of rickets? (2) What is the proper dosage? (3) What are the effects of its prolonged use? (4) What is the margin of safety between the therapeutic and toxic dose? (5) What are the factors which predispose to toxic effects, if any? (6) What are the indications for and contraindications against the use of viosterol? Although some of these questions have been, in part, clarified by animal experimentation and clinical observation, many controversies remain. Some of these may be attributed to the use of irradiated ergosterol of doubtful potency. Thus, much of the earlier work on the clinical and experimental use of irradiated ergosterol was based on milligram dosage; and one milligram of the substance may have contained little or no vitamin D, or a content several hundred times that of cod liver oil. In many instances, the amounts administered were variable, the conditions under which viosterol was used were not always satisfactory, the degrees of rickets were not clearly classified, and the infants were not always followed long enough; they differed widely in age, diet, and environment, or were of different race, or the total number was too small.

The present study, begun in March 1929, covers a period of over six years.

2. AIM OF THE STUDY

The main objects of the study were to determine:

(a) The relative dosage of viosterol in the prevention of rickets in (1) white children, (2) colored children, (3) premature babies and twins.

(b) The dosage of viosterol necessary to cure completely various degrees of active rickets and, incidentally, (1) the relation of size of dose to time required for a complete cure and (2) the comparative value of slow versus rapid healing.

(c) The effect of viosterol in infantile tetany.

(d) The effect of viosterol in late rickets, celiac disease, the healing of fractures, etc.

(e) The effects of various dosages of viosterol on the concentration of serum calcium and inorganic phosphorus.

3. PLAN AND CONDUCT OF STUDY

Clinical material. The babies included in the prophylactic group were from the wards and dispensary of the Harriet Lane Home of the Johns Hopkins Hospital and from the Welfare Clinics of the City of Baltimore. The infants with active rickets and tetany were either bed- or out-patients.

(a) In the prophylactic group, the children had not previously received vitamin D. The viosterol was given in specified doses, ranging from 5 to 20 drops daily. The children from the Welfare Clinics were examined periodically by the physician-in-charge, and frequent home visits were made by nurses attached to the clinics and also by our own social service workers. Those from the Harriet Lane Home wards or dispensary were seen periodically by the house or dispensary staff and a record was kept of the children's progress in nutrition and health. Frequent home visits were also made by a social worker assigned solely to this investigation, to ascertain whether or not viosterol was being administered regularly. A rough index as to the regularity of viosterol feeding could be obtained by noting the time in which a bottle of viosterol was consumed. X-ray examinations of the extremities were made about every three months and the interpretations were made by at least three persons—the final judgment, however, resting with the senior author. Serum calcium and inorganic phosphorus determinations were made whenever feasible.

(b) In cases of active rickets x-ray films of the bones were made before antirachitic therapy was instituted, and, in many cases, the serum was analyzed for calcium and inorganic phosphorus. X-ray films of the bones were obtained at intervals until the rachitic process had entirely healed. When possible, serum calcium and inorganic phosphorus determinations were made at different times during the healing periods. The infants discharged from the wards, as well as those in the dispensary, were followed through the aid of a social service worker specially assigned to this study, whose intelligent supervision resulted in excellent coöperation on the part of the mothers, thereby assuring regularity in the administration of the viosterol.

The uncertainty of administration of cod liver oil did not prevail in the case of viosterol.

(c) The infants with tetany were either studied on the wards or followed in the dispensary and at home at very frequent intervals. In most instances, calcium preparations were given to alleviate the immediate tetanic manifestations and the viosterol was continued until all evidences of tetany, or of rickets and tetany, had disappeared.

4. CRITERIA FOR THE DIAGNOSIS OF RICKETS AND TETANY

The means employed for the diagnosis of rickets were: Clinical examination, calcium and inorganic phosphorus determinations in the blood serum, and roentgenological examination of the bones. The last was found to be the most reliable method.

On examination of the child, special attention was paid to the clinical signs indicative of rickets, such as, craniotabes, sizes of fontanels, shape of chest, the degree of beading of the costochondral junctions, pot belly, and the degree of enlargement of the wrists and ankles. In many younger infants, record was made of the state of the muscular and nervous development and of dentition. In older children, the enlargement of the wrists and ankles and deformities of the extremities and of chest were not considered as evidence of active rickets unless confirmed roentgenologically, since, in many instances, these signs are merely indicative of preexistent rickets. The physiological enlargements of the wrists, particularly in fat babies, angulations of the costochondral junction, and soft chests were not considered as evidence of rickets unless shown to be rachitic by x-ray films. In the curative group, therefore, no infant was considered to have rickets, and hence, to be suitable for study, unless the clinical, roentgenological and the chemical findings, if the last were known, were confirmatory. In the prophylactic group, rickets was not excluded or diagnosed unless made certain roentgenologically.

The criteria for the diagnosis of tetany were the usual Chvostek and peroneal reflexes, carpo-pedal spasm, and convulsions. In many instances, the diagnosis was corroborated by the finding of lowered concentrations of calcium in the serum; and in a very few, by the demonstrations of the characteristic lowered galvanic excitability.

5. STANDARDIZATION OF VIOSTEROL USED IN THIS INVESTIGATION

The antirachitic potency of the viosterol[1] used in this study was determined by the manufacturer and frequently used in rat experiments and in that way tested by us. It was maintained throughout at a potency of 100 D. The designation "D," however, has been recently abandoned, and all vitamin D preparations are expressed in International Units. In the International nomenclature, the potency of one gram of *average* cod liver oil is expressed as 100 units, and that of viosterol as 10,000 units, a ratio of 1:100. *In this study the viosterol used is considered to have 100 times the antirachitic potency of "good" cod liver oil.*[2]

6. THE CLASSIFICATION OF CASES OF RICKETS ACCORDING TO THE SEVERITY OF THE DISEASE

The clinical and x-ray criteria of rickets are susceptible of subjective interpretation. Unless there is agreement in regard to the extent and severity of the rachitic process, no two reports are comparable and no definite conclusions can be arrived at in regard to the efficacy of a given dose of an antirachitic agent. As previously mentioned, the radiographic appearance of the bones was chosen as the chief criterion, and it was likewise used as a means of determining the *activity* of the rachitic process. Typical roentgenograms illustrating rickets of varying severity are shown in Figure 1. The degrees were designated as (1) *mild*, (2) *moderate*, (3) *severe*, and (4) *very severe*. In the mild cases, the most common seats of activity detectable roentgenologically were the distal ends of the radius and ulna, both ends of the tibia and fibula, and the distal end of the femur. In the very severe cases, most

[1] Generously supplied by Mead Johnson and Company, Evansville, Indiana.

[2] The following calculations show the relative antirachitic potency, expressed both in Steenbock and International units, of average cod liver oil and of viosterol.

(a) 1 teaspoonful cod liver = 4 cc. (average) = 135 Steenbock or 400 International units. (1 cc. oil = about 0.9 gm.)

(b) 3 teaspoonfuls cod liver oil = 400 Steenbock or 1,200 International units.

(c) 1 cc. viosterol = 40 drops = 3,000 Steenbock or 9,000 International units.

(d) 1 drop viosterol = 75 Steenbock units or 225 International units.

(e) 5 drops viosterol = 375 rat units or 1,125 International units.

(f) ∴ 3 teaspoonfuls cod liver oil = 5 drops of viosterol.

of the bones of the body were involved, including the scapula and pelvic bones. In some of the latter cases, the rachitic process was so advanced that the structure of the bones appeared extremely porotic and the shafts showed numerous fractures (figures 1–D, 11, 12, 16 and 18).

The *healing* brought about by viosterol, at varying periods after its administration was begun, was classified as: (1) *beginning*, *i.e.*, when

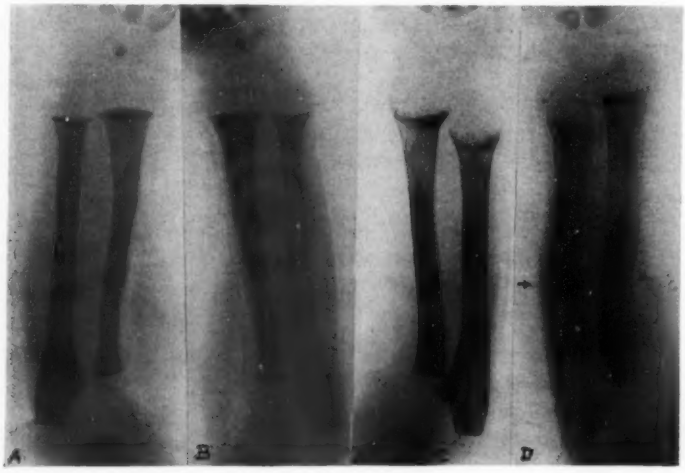

FIG. 1. ILLUSTRATING THE VARIOUS DEGREES OF RICKETS AT THE DISTAL ENDS OF THE RADIUS AND ULNA: *A*, MILD; *B*, MODERATE; *C*, SEVERE; *D*, VERY SEVERE

Arrows indicate sites of fractures and of periosteal reaction

lime salts began to be deposited at the cartilage-shaft junction, (2) *advanced*, when the ends of the bones, including the rachitic area, became nearly completely impregnated with lime salts, and (3) *complete* or *healed*, when the ends of the bones assumed an approximately normal definition and appearance.

In the prophylactic group, no infant was considered to have active rickets unless both the clinical and roentgenological signs justified such as a diagnosis. The physiological cupping seen in the roentgenograms

of the ends of the bones was not mistaken for rickets, since the architecture of such bones is normal, the metaphyses are not widened, and the ends are well calcified.

7. EFFECT OF VIOSTEROL IN THE PREVENTION OF RICKETS

Most of the infants of our study received 5 or 10 drops of viosterol daily and but a small number were given 15 or 20 drops. The larger doses were given to twins, prematurely born or rapidly growing infants, and poorly nourished congenital syphilitic babies.

In the past two years we were able to study a group of infants receiving only 5 drops of viosterol daily. The group consisted of 42

TABLE 1

Showing the distribution of cases in the prophylactic group, according to race and dosage of viosterol, and the percentage of protection against rickets in each viosterol group

DROPS OF VIOSTEROL DAILY	NUMBER OF PATIENTS		TOTAL PATIENTS IN EACH GROUP	PER CENT PROTECTED
	Colored	White		
5	133	42	175	100* (98.3)
10	66	207	273	100
15	21	23	44	100
20	15	18	33	100
Total cases....	235	290	525	100

* Three of the colored patients in this group showed, after three to four months of therapy, radiographic evidence of mild rickets which subsequently disappeared on the same dosage of viosterol.

white and 133 colored infants, most of whom were started on viosterol during the first month of life and kept on the same amount for nine months or longer. The inclusion in this group of a large number of very young colored infants is of considerable significance, since they are most susceptible to rickets.

The accompanying tables show the distribution of the groups according to: (1) dosage of viosterol, (2) race, (3) age at the beginning of therapy, and (4) duration of viosterol administration. There were 525 infants in the entire group—290 white and 235 colored. From tables 1 and 2, it is seen that most of them began taking viosterol when they were under five months of age and continued to take it for

TABLE 2

Distribution of cases in the prophylactic group according to race, dosage of viosterol, age at beginning of viosterol administration, and duration of therapy

The numbers in each square indicate the number of subjects started on viosterol at the month shown in the ordinates and continued for a period of months indicated in the abscissae. For example, in the group receiving 10 drops of viosterol, the numeral 8, in the second line above the figure 15 in the abscissa, represents 8 cases started on viosterol in the second month of life and continued for fifteen months.

20 drops viosterol daily: Total cases, 33; White, 18; Colored, 15

10							3					
						1						
				1								
	1						1					
							1	3				
5	1							1		1		
	1	2							1			
		1					2		1			
1		1	1			3	4	1	1			

5 drops viosterol daily: Total cases, 175; White, 42; Colored, 133

5							5		1			
			2			3	1	2				
			3	2	1	1	4	3	1			
	6	8	13	5	4		8	13	9	1		
1	3		13	7	2	5	11	19	11	4	2	
	6			10					15			

15 drops viosterol daily: Total cases, 44; White, 23; Colored, 21

			1																	
5		1							1											
									3											
		1	1				1		1	1										
		1	2		1		1	3	2	1	1						1		2	
1				1	1	2	1	3	7		1	2								
		6				10				15					20				24	

10 drops viosterol daily: Total, 273; White, 207; Colored, 66

	1			1			1	1																	
					1																				
					1																				
10								1	1		1														
			2																						
											1														
	2		2			1	1	1	1	1	1														
	3	1		1			1	1	2	2	1					1									
5	3	3	1	1	1	1	5	3		3	1						1		1						
	3		3	2	2	1	9	4	1		3	1						2		1					
	7	5	11	6	3	6	11	14	13	8	2	5	6	3	1	1	1		1	1					
1	5	2	5	8	4	2	14	13	10	4	1	3	5	1											1
	6			10				15				20				25				30					

Ages viosterol begun—months

Duration of viosterol administration—months

nine months or longer. Of the total number of 525 infants, 273 received 10 drops and 175 received 5 drops daily—a total of 448 or 85.3 per cent. The remaining 77 received the larger doses. Of the "10 drop" group, 66 were colored and 13 were Italians; and of the "5 drop" group, 133 were colored.

The results with the prophylactic use of viosterol as an antirachitic agent were, on the whole, gratifying. The children receiving 10 or more drops, including the colored children, *failed to show clinical or roentgenologic evidence of rickets.* Indeed, the x-ray films of the long bones of many of the white children and of some of the colored children, receiving 10 drops of viosterol daily for nine months or longer, showed bright terminal zones, indicative of superior calcification. An example of such calcification of the long bones of a white child who received 10 drops of viosterol daily for fifteen months, beginning at one month of age, is shown in figure 2–A, and that of a colored infant receiving the same amount of viosterol for fifteen months, beginning at four months of age, in figure 2–B. In the group receiving only 5 drops of viosterol daily, the 42 white children likewise remained free; 3 of the 133 colored children, however, showed the slightest degree of rickets shortly after the viosterol administration was begun but no evidence of the disease at the end of the study period (table 2). Considering the poor hygienic and nutritional conditions of the negro population in Baltimore and the susceptibility of the colored infants to the development of rickets, the prevention of rickets in this group by as little as 5 drops of viosterol daily seems to us to be highly significant. The x-ray appearance of the bones of two colored children who received only 5 drops of viosterol daily for nine and twelve months, respectively, beginning at the age of nineteen and forty-seven days, are shown in figures 2–C and D.

Further proof that this amount of viosterol is capable of preventing rickets in colored children was obtained by examining histologically the bones of a colored female so treated from one month of age until fourteen and one-half months, when she died of pertussis encephalitis. The histologic appearance of one of her ribs, showing no evidence of rickets, is illustrated in figure 3. Several bones, obtained at autopsy, of children receiving larger doses of viosterol likewise showed normal structure and, in some instances, superior calcification at the cartilage-shaft junctions. An example of normal histologic and

x-ray appearances of a bone from a ten-month-old child receiving 20 drops of viosterol for five months is shown in figure 4.

It is interesting that many of the colored children in the prophylactic group who were protected against the development of rickets with as little as 5 drops of viosterol daily came from families in which other members had been treated by us for active rickets.

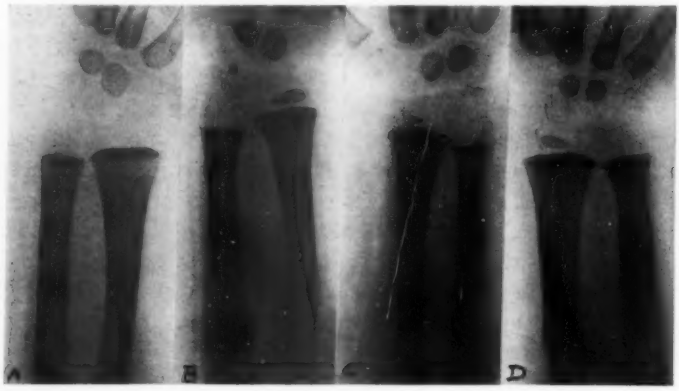

FIG. 2. *A*, X-ray appearance of the bones of a white infant who received 10 drops of viosterol daily for fifteen months, beginning at one month of age. *B*, X-ray appearance of the bones of a colored child given 10 drops of viosterol daily from the fourth to the nineteenth month of life. Note the dense terminal zones of the radii and ulnae, indicating superior calcification. *C* and *D*, Normally appearing bones of two colored infants fed only 5 drops of viosterol daily beginning at nineteen and at forty-seven days and continued for nine and twelve months, respectively.

Our results with the prophylactic group are apparently not entirely in agreement with those reported by DeSanctis and Craig (1) and by Barnes *et al.* (2). The former investigators found that 3 teaspoons of medicinal cod liver oil daily prevented rickets in 97 per cent of the 100 cases studied, whereas 10 drops of viosterol 100-D daily prevented rickets in only 77 per cent of 123 cases, 23 per cent developing *clinical* signs of rickets. The latter reported that 3 teaspoons of Norwegian cod liver oil prevented or cured rickets in 95 per cent of the cases studied, whereas 125 Steenbock rat units of viosterol prevented

rickets in only 44 per cent of the cases. The apparent disagreement *may* perhaps be explained on the basis of differences in the conduct of experiments. It must be appreciated that the experiments of both sets of investigators were conducted early in the developmental period of viosterol therapy, at a time when criteria for the conduct of such investigations were as yet not established and methods for the

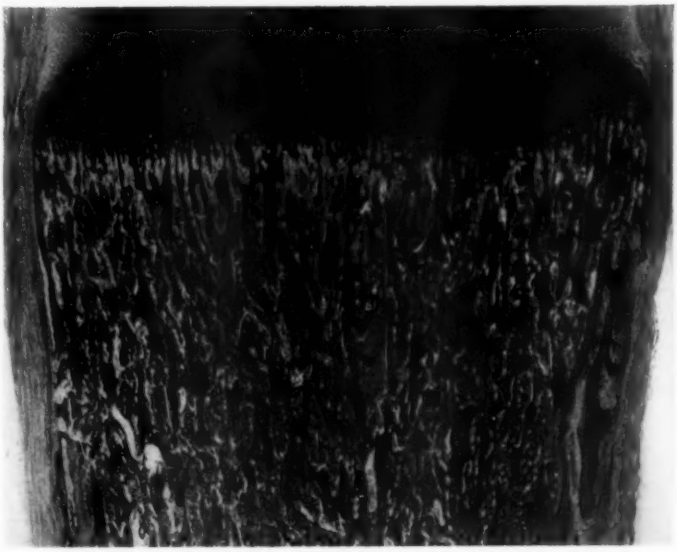

FIG. 3. HISTOLOGIC APPEARANCE OF THE RIB OF A COLORED CHILD WHO RECEIVED 5 DROPS OF VIOSTEROL DAILY FROM THE AGE OF ONE MONTH UNTIL HER DEATH FROM PERTUSSIS ENCEPHALITIS AT FOURTEEN AND ONE-HALF MONTHS

Note the orderly arrangement of the cartilage zone and the well calcified trabeculae

assay of vitamin D varied considerably from the "official" method now universally accepted.

Referring first to the study of DeSanctis and Craig, the infants in neither the "cod liver oil" nor the "viosterol" group were divided as to race; they were followed for short periods; they were not supervised sufficiently to be sure that the antirachitic agents were administered

with equal regularity; the viosterol employed was of 3 different brands;[3] the presence or absence of rickets was based entirely on the clinical opinions without confirmation by roentgenograms.

The diagnosis of rickets by clinical opinions alone is not without its limitations. Palpable beading of the costochondral junctions was used by DeSanctis and Craig as the chief criterion for the diagnosis of

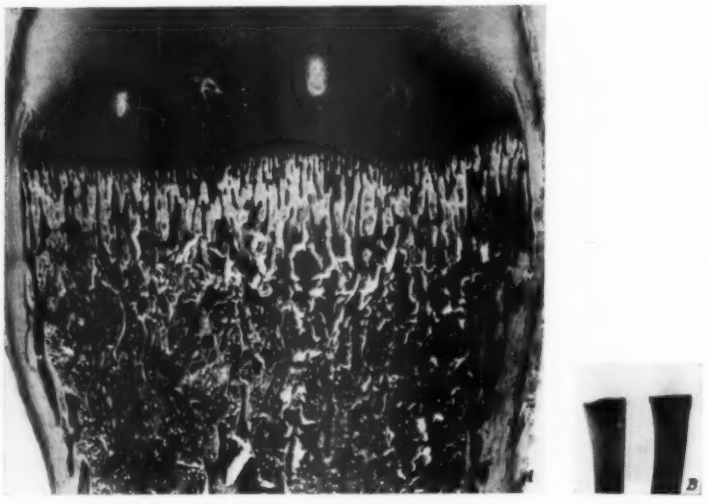

FIG. 4. ILLUSTRATING THE NORMAL HISTOLOGICAL AND ROENTGENOLOGICAL APPEARANCES OF THE BONES OF A TEN-MONTH-OLD WHITE CHILD RECEIVING 20 DROPS OF VIOSTEROL DAILY FOR FIVE MONTHS

Clinical history: Unexplained convulsions for six months; refractory to therapy. Died in convulsions. *Autopsy:* No lesions found.

rickets in 27 out of 29 cases in the viosterol group, but the fact that angulation of the ribs in apparently normal infants is frequently mis-

[3] Personal communication. Regarding the potency of the viosterol employed, Holmes and Piggott (3) found that of the 6 samples of 5 brands on the market, tested by them in the winter of 1928–29, three contained little or no antirachitic potency. The greater incidence of rickets in the viosterol group may thus have been due, not so much to the inefficiency of the viosterol *per se*, as to the inadequate concentration of vitamin D in one or more of the preparations.

taken for rachitic rosaries renders the diagnosis of active rickets in their 27 infants somewhat doubtful.[4] In a recent communication, Park and Eliot (6) state: "In moderate and advanced rickets the enlargements are so marked as to leave no doubt as to their nature. The greatest difficulty is to differentiate by means of examination of the costochondral junctions slight rickets from the normal. No method exists by which the enlargements can be measured and exact information obtained. The examiner is forced to depend on the impression derived from the sense of touch that the costochondral junction is larger than it should be, but this impression is apt to be wrong . . . The diagnosis of rickets should never be made from the enlargement of the costochondral junctions alone."

That there is a greater tendency to make the diagnosis of mild rickets *clinically* when the disease actually is not present, than fail to recognize the disease when it *is* present, is becoming more evident from statistical studies on large numbers of children. In a study of 956 negro and Italian children in New Haven, Eliot and Jackson (7) found that only 37 per cent of the clinical diagnoses of rickets could be corroborated by roentgenographic examination; and in another investigation of 586 children in Puerto Rico, Eliot (8) discovered a similar but less pronounced inconsistency between the clinical and roentgenographic signs of rickets. This fact is also illustrated in the cases studied by Hess, Poncher, Dale and Klein (9). From their tables it may be seen that in many instances in which rickets was diagnosed clinically, the x-ray films of the bones failed to substantiate the clinical impressions.

[4] Moreover, according to Irish (4) and Hess (5) beading of the costochondral junctions may actually indicate an excess deposition of lime-salts rather than the presence of rickets. Hess calls attention to the fact that "in examining infants for rickets, it should be borne in mind that there is not only a rachitic beading but also what may be termed antirachitic beading. By this is meant an enlargement of the costochondral junctions due to giving antirachitic agents for long periods and especially in excessive amounts. Beading of this kind may be mistakenly judged as rachitic in nature and may lead to the inference that the therapy has been ineffective. Associated with the 'antirachitic beading,' one may find hypertrophy of the epiphyses of the wrists, associated with hypercalcification but unaccompanied by any changes in the calcium or phosphorus concentration of the blood."

In our own clinic, where rickets is a very common disease and the physicians are accustomed to seeing a great number of rachitic children, the clinical diagnoses of mild or moderate rickets are not always confirmed radiologically. Park and Eliot (6) speak of this discrepancy as follows: "It is necessary to issue a warning, because the diagnosis of rickets is so frequently erroneously made in normal children as a result of both clinical and x-ray examinations. . . . *The greatest difficulty is to recognize rickets when it is only slightly developed and to abstain from diagnosing early rickets in normal infants.*"

With respect to the investigation of Barnes *et al.*, the high incidence of rickets in their "irradiated ergosterol" group may be attributed to several factors. First, the dosage of irradiated ergosterol[5] used was quite inadequate—125 Steenbock rat units (340 I.U.) daily—and hence its failure to protect very young, rapidly growing colored infants against the development of rickets is not at all surprising, since a similar number of rat units of cod liver oil or other fish oils would probably also have been ineffectual. Secondly, the children in the cod liver oil group received about five times as much vitamin D as those in the viosterol group. Good grades of Norwegian cod liver oil, as sold on the open market in 1928–29, contained, on the average, 37 Steenbock or 100 International rat units per gram.[6] On this basis, the 3 teaspoons of such oil as was used by Barnes and others probably contained about 400 Steenbock or 1200 International rat units. Thirdly, "No attempt was made to select the infants. They were taken as they came into the clinic and put in rotation in one of the four groups." This procedure led accidentally to a probable error, especially with regard to such factors as race and age. Thus, when their table 1, the "viosterol group," and table 2, the "cod liver oil group," are compared, it becomes evident that, although in the viosterol group the percentage of colored infants is only somewhat higher than in the cod liver oil group (78.5 as compared to 69 per cent), yet the percentages of infants six months of age or under and of those

[5] The preparation used by Barnes *et al.* was not commercial viosterol but one prepared by them especially for that study.

[6] The figures for the vitamin D potency of the cod liver oil used by Barnes *et al.* were obtained from the distributors who supplied the oil and from several research workers who tested the oil for its antirachitic potency.

ten months or over differ quite markedly, as may be seen in the recalculated table 3. It shows that of the total infants in the viosterol group 63 per cent were six months of age or under, whereas in the cod liver oil group the percentage was 30. Since the incidence of rickets is greatest between the fifth and twelfth months of life (fig. 8), the lower incidence of rickets in the cod liver oil group may have depended not so much on the superiority of cod liver oil as on differences in age. It is unlikely that Barnes, Brady and James' patients 211, 213, and 1,007 would have developed rickets, since the ages of these *white* infants were twelve and thirteen months and the periods of observation were only 85, 81 and 98 days respectively. Reference to their table 4, the control group, receiving no vitamin D, confirms this belief,

TABLE 3

Recalculations from table 1 (viosterol group) and table 2 (cod liver oil group), according to race and age

(From Barnes *et al.*)

TOTAL, ALL CASES	UNDER 6 MONTHS OF AGE				10 MONTHS OR OLDER			
	Total	Colored	White	Per cent for entire group	Total	Colored	White	Per cent for entire group
Viosterol, 57	36	29	7	63.0	10	5	5	17.5
C. L. O., 64	29	24	5	45.2	21	12	9	30.0

as none of the white children and but a few of the colored children past the age of ten months developed rickets.

Our results with regard to the efficacy of viosterol as a prophylactic against rickets are in agreement with those of Hess, Poncher, Dale and Klein (9) in this country and those of numerous investigators abroad. The results of the European studies have been summarized by György (10), Aengenendt (11), Schaferstein (12), and by others. Most of the European studies, however, are not strictly comparable to those conducted in the United States and Canada, since in the former the irradiated ergosterol was administered, until recently, in milligram doses, instead of "rat units," as is the custom on this continent. The more recent study of Soer (13), of Holland, is one of the exceptions. He used irradiated ergosterol standardized according to the adopted International procedure and found that as little as 400 International

units (135 Steenbock units) were sufficient to prevent rickets in 77 out of 80 children and that twice this amount protected the entire group of 36 infants. These amounts of irradiated ergosterol are even smaller than the minimal doses employed by us.

The most noteworthy American studies on the effects of standardized viosterol in the prophylaxis of rickets are those of the late Alfred Hess and his associates. This group has contributed numerous papers dealing not only with the effects of irradiated ergosterol but also with the prophylaxis of rickets with vitamin D milk produced under various conditions, such as direct irradiation of the milk or of the cow, or the feeding to the cow of substances containing vitamin D. However, their reports with regard to dosage of viosterol are rather confusing. In 1929, soon after standardized viosterol was introduced on the market, Hess, Lewis and Rivkin (14) recommended 8 to 10 drops of viosterol 100-D as a prophylactic dose for full-term infants growing at a normal rate. In 1930, they (15, 16) stated that three to four times as much irradiated ergosterol was necessary to protect infants against rickets as cod liver oil having an equal number of rat units, and, accordingly, they increased the prophylactic dose of viosterol to as high as 30 drops daily. However, the confusion with regard to the relative antirachitic potencies of cod liver oil and viosterol was largely due to the fact that the potency of the so-called Steenbock or American Medical Association "standard" cod liver oil was 2.8 to 3.0 times weaker than ordinary cod liver oil which contains, on the average, 37 Steenbock or 100 International units. Hess's comparison was apparently based on the assumption that the Steenbock or the American Medical Association "standard" was correct and hence he inferred that the cod liver oil which he himself used contained 13.3 Steenbock units (36 International units) per gram[7] when there is every reason to suppose that it contained the usual 37 Steenbock or 100 International units. Thus, when Hess estimated the potency of viosterol in terms

[7] The statement "We know that 1 gm. of standard cod liver oil contains 13.3 rat units" (16), made by the late Dr. Alfred F. Hess in 1930, was based, according to Lewis [personal communication], on computations in terms of the American Medical Assn. "standard" cod liver oil rather than on actual analyses of the oils used. In 1933, Hess and Lewis (5) stated that average cod liver oil contains from 37 to 40 Steenbock rat units.

of cod liver oil, the potency of which was underestimated, at least a threefold undervaluation of viosterol resulted.

From our study, it would appear *that 5 drops of viosterol is the dose which will protect most full-term white and even colored infants.* As we have not determined by systematic study the preventive dosage of cod liver oil in a similar group of children, we are unable to state definitely how the antirachitic efficacy of viosterol compares with that of cod liver oil containing a similar number of rat units of vitamin D per dose. The experience of others is also of little aid in determining this point, since the opinions of clinicians vary quite considerably as to what constitutes a daily prophylactic dose of cod liver oil in infants, as may be inferred from the replies to a recent questionnaire sent out by the Council on Pharmacy and Therapeutics of the American Medical Association (17). In the replies from well-known clinical observers, the daily dosages ranged from about 0.1 of a teaspoonful to 5 teaspoonfuls of the oil daily—the disagreement probably being due to the fact that not all clinics deal with the same racial groups or are located in similar climates. Under the conditions prevailing in Baltimore, New York, and New Haven, Park (18), Hess (19), and Eliot (20) recommend at least 3 teaspoonfuls of cod liver oil, or an amount which usually assays 400 Steenbock or 1200 International units, as the daily prophylactic dose for Negro children. This dose is practically the same in antirachitic potency as 5 drops of viosterol (1125 International units)—an amount sufficient to protect even colored infants against the development of rickets. In exceptional cases, rickets may develop on any form of therapy, but perhaps more often on cod liver oil than on viosterol, largely because the disagreeable taste and odor of the former contribute to laxity in its administration. We believe, however, that the only superiority of viosterol over cod liver oil is in the ease with which viosterol is taken, and, hence, in the greater probability that the child will receive it. Our experience with antirachitic agents leads us to concur with the opinions expressed by Hess (16) that it is easy to prescribe cod liver oil but extremely difficult to be certain that it is taken regularly.

At this juncture, we wish to call attention to a frequent error in the technique of the administration of vitamin D, namely, the addition of viosterol to the milk formula—a procedure which Hess (19) recom-

mended for cod liver oil administration—instead of giving it separately or in a spoonful of orange juice. It must be remembered that, unless an oil is "homogenized" or emulsified mechanically, it will not mix with milk or other aqueous solutions but will float on the top and adhere to the sides of the container, and the infant may thus fail to receive the recommended amount of vitamin D.

8. THE EFFECT OF VIOSTEROL IN THE PREVENTION OF RICKETS IN PREMATURE INFANTS, TWINS, AND LUETIC INFANTS

According to Hamilton (21), 85 per cent of the calcium supply of the newly born infant is stored during the last two to three months of intrauterine life. *In utero*, this supply is derived from maternal sources; but in the prematurely born infant, it must be obtained from the food. The accelerated skeletal deposition of lime salts during the early post-natal existence of the premature infant naturally requires a greater supply of calcium and phosphorus; and unless this added requirement is provided for, the child may develop rickets or osteoporosis. The susceptibility of premature infants to rickets may also be attributed to the rapidity with which these infants grow in their two or three months of post-natal life. According to Park (22), "Rickets cannot occur without growth, because lime salts cannot fail to deposit unless newly formed tissue exists for them to fail to deposit in"; and this fact is now fully appreciated by those engaged in the study of rickets in human beings and in experimental animals. It would appear that, although rickets may occur in poorly nourished colored infants because of an actual paucity in the intake of lime salts, the severer types of the disease in both infants and experimental animals usually develop in the well-fed and in those gaining weight at a rapid rate. The susceptibility of premature infants to rickets is, therefore, due to the greater requirement for calcium phosphate, to the rapidity of skeletal growth, and possibly also to the utilization of large portions of the phosphorus ingested in building rapidly-growing soft tissues. It is also possible that premature infants are unable to utilize calcium phosphate, even when it is supplied in seemingly optimal amounts, unless larger doses of vitamin D are given.

Because of the predisposition of premature infants to rickets their requirement for vitamin D is also greater than that of full-term babies.

From our own observations and those of others, it appears that the usual dose of cod liver oil—3 teaspoonfuls—may fail to protect against rickets in prematures, especially of the colored race; and since larger amounts of cod liver oil are difficult to administer without causing regurgitation or gastro-intestinal upsets, the high concentration of vitamin D in viosterol makes it an ideal source of the vitamin for prematurely-born infants.

We have studied the efficiency of viosterol in a number of prematurely-born infants, observing them not only during the first few months of life but for longer periods. In most of the infants, the viosterol feeding was begun during the first month of life, and in the majority it was continued for six months or longer. In addition to the

TABLE 4

Showing the distribution of the 56 cases of prematurity according to race, dosage of viosterol, and percentage protection

VIOSTEROL DOSAGE	RACE		NUMBER INITIALLY NOT PROTECTED*		NUMBER FINALLY PROTECTED*		PERCENTAGE PROTECTION
	Colored	White	Colored	White	Colored	White	
drops							
5	2	1	2	0	2	1	100*
10	10	15	3	0	10	15	100*
15	9	6	0	0	9	6	100
20	7	6	0	0	7	6	100

* See text.

56 infants analyzed in table 4, 23 were followed for shorter periods of three to five months, thus making a total of 79. Of the 56 infants, 28 were white and an equal number were colored. Most of them received either 10 or 15 drops, a smaller number received 20 drops, and only 3 received 5 drops of viosterol daily. The dosages of viosterol used in each racial group and the number protected in each viosterol group are shown in table 4. In analyzing the results of this study, cognizance was taken of the factor of age. Accordingly, the effect of viosterol in preventing rickets in premature infants was studied in the same infants during the period of rapid growth—the first three or four months of life—and also at later periods. Viewing the results from the later periods alone, the impression would be gained that

premature infants differ but little from full-term babies in their susceptibility to rickets and in their response to vitamin D, since the x-ray films of the long bones of the entire group, taken at the later dates, failed to show rickets. When, however, the earlier x-ray films were examined, it was found that the 2 colored infants in the groups receiving 5 drops and 3 colored infants in the group receiving 10 drops of viosterol daily had very mild fraying and fuzzing at the distal ends of the femora, radii and ulnae—radiographic signs which might be interpreted as early rickets. A few of the radiograms showed only lateral spurs at the distal ends of either the radii or ulnae, and no other signs of rickets. The fact that these signs of deficient ossification were absent in subsequent x-ray films indicates the mildness of these rachitic changes, and also shows that, if viosterol is taken regularly, even in moderate doses, the chances of developing *active* rickets are very small. These early evidences of rickets do demonstrate, however, that it might be desirable to give vitamin D in doses somewhat larger than the average during the first four or five months of life of premature babies, and that supplementary calcium phosphate during that period might be advisable, regardless of the amounts of vitamin D taken. The last suggestion is based on the fact that in some of the infants, in whom there were no radiographic signs of rickets at the growing ends of the bones, there was some evidence of osteoporosis—a derangement in ossification which is due to an actual paucity in calcium and not necessarily to an inadequacy in vitamin D. The statement made by Davidson and Merritt (23) that osteoporosis *per se* is a sign of rickets in infants is not borne out by histologic examination of the bones of infants or by experimental observations on animals. In the rat, for example, the feeding of a diet low in calcium may produce osteoporosis without rickets, the porosity of the bones being present in spite of the administration of usual amounts of vitamin D (24). It is due, in all probability, to an actual deficiency in the intake of lime salts; and no amount of vitamin D is sufficient to prevent this disorder of ossification, unless calcium is provided at the same time.

In a recent communication, Davidson and Merritt (23) described what they consider to be a "hitherto undescribed" early sign of rickets in premature infants, namely, "marked deposition of uncalcified bone

at the proximal medial surface of the shaft of the ulna," characterized by "a frayed or chewed-out, ragged appearance" of the bone. Reference to their x-ray films shows that, in those in which this defect is present, there already existed definite evidence of rickets in the distal ends of the radius and ulna, and, therefore, the changes at the proximal ends are in reality later manifestations of the disease. The question as to whether or not the proximal end of the ulna is or is not the site of the first signs of rickets would be of little significance, had it not been for the importance placed on it by Davidson and Merritt in their studies which resulted in the conclusion that viosterol, even in dosages of 20 drops daily, was inadequate as a protection against rickets in premature babies.

Since the idea that the proximal end of the ulna is the site of the earliest signs of rickets in premature infants is novel, and since the conclusions of Davidson and Merritt with respect to the inefficacy of viosterol in the prevention of rickets in their 13 premature infants depended to some extent on this sign and are not in accord with our own findings, we rechecked our results in several ways: (1) by areexamination of the x-ray films of the bones of treated and untreated premature infants, taken during their first three months of life, with a view to discovering the presence of early signs of rickets at the proximal ends of the ulna which we might have overlooked; (2) by a similar examination of the x-ray films of the bones of premature infants who came to autopsy; and (3) by an histologic study of the bones of the latter group.[8] We have been fortunate to find, among the autopsy cases, 43 instances of prematurity in which the bones had been examined by roentgenograms and by direct histologic examination. Some of these premature infants, dying at an early age, were given either viosterol or cod liver oil; others received no vitamin D at all. In many instances, death occurred between the second and third month of life, *i.e.*, during the period at which one would expect to find the earliest sign of rickets in the proximal end of the ulna, had it been present.

In the roentgenograms of the bones of the living children, signs of

[8] The latter data were obtained through the kindness of Dr. E. A. Park and Miss Deborah Jackson, whose collection of bones from young infants, started in 1928, is now derived from about 1200 autopsies.

rickets at the medial side of the proximal end of the ulna were found only when fairly advanced signs of the disease were present in other sites. Indeed, many of the cases showing definite evidence of rickets in the distal ends of the radius and ulna failed to show any changes

TABLE 5

Histological and roentgenological findings in the bones of fourteen cases of prematurity receiving viosterol as a prophylactic against rickets during the periods of most active growth

SECTION NUMBER	NAME	RACE SEX	AGE	VIOSTEROL	DURATION	POST-MORTEM X-RAY	HISTOLOGY	COMMENT
			mos.	*drops*				
224	J. W.	WM	4	10	42 days	Negative	Negative	
417	A. B.	WF	12	20	11 months	Negative	Negative*	
466	C. C.	CM	4	20	6 weeks	Negative	Negative	See figure 5–C
				10	9 weeks			
500	D. A.	CF	2	10	4 weeks	Negative	Negative	Hydrocephalic
540	G. B.	CF	3	10	14 days	Negative	Negative	
				15	39 days			
				20	32 days			
543	D. S.	CF	2.5	15	33 days	Negative	Negative	See figure 5–B
765	W. T.	CM	1.5	20	24 days	Negative	Negative	
802	M. G.	CF	3	15	21 days	Negative	Negative	
				25	12 days			
905	T. M.	CF	11	20	11 months	Negative	Negative	See figure 5–D
958	A. E.	CM	2.6	10	1 week	Negative	Negative*	
				15	1 week			
959	J. R.	WM	2.5	10	14 days	Negative	Negative*	
963	J. D.	WF	2	5	7 days	Negative	Negative	See figure 5–A
				10	7 days			
				15	39 days			
988	R. S.	CF	10	30	6½ months	Negative	Negative	
				15	3½ months			
1003	G. W.	CF	2.5	10	6 months	Negative	Negative	
				15	58 months			

* These were examined under a high-power dissecting microscope without recourse to histologic sections.

at the upper ends of these bones. In the roentgenograms of the bones obtained at autopsy, the findings were identical. Of the 23 cases, in which radii and ulnae were obtained, conspicuous changes in the proximal end of the ulna were absent in 11 cases showing some degree

of rickets in one or more bones, and only definitely present in one case and suggestive in 4 other cases of florid rickets.

The histologic examination of the bones also failed to disclose the presence of rickets at the upper end of the ulna when it was absent elsewhere, or its appearance at this site without being also present, in a more advanced state of development, in the more rapidly growing ends of the long bones.

This study also offered an opportunity to investigate, histologically, the efficacy of viosterol in the prevention of rickets in prematures and to compare the findings with our clinical and roentgenological observations. The results of this study are shown in table 5. Of the 14 infants receiving viosterol from about the first few weeks of life until shortly before death, 10 were between two and four months of age at the time of their demise, 2 died at ten months of age, and the remaining 2 died at eleven and twelve months, respectively. In none of these was rickets present roentgenologically or histologically, in spite of the fact that these children, mostly colored, had received only 10 to 20 drops of viosterol daily. The histologic appearances of bones of premature infants receiving 10 to 20 drops of viosterol daily for from thirty-three days to eleven months are shown in figure 5.

The x-ray appearances of the radii and ulnae of two living colored premature infants, receiving daily doses of 10 and 15 drops of viosterol, respectively, from the first week of life until one year of age, are shown in figures 6 and 7. The radiographs shown in figure 6–A and B were taken after eight weeks and one year of therapy with 10 drops of viosterol daily, and those in figure 7–A and B were taken after six weeks and one year of treatment with 15 drops of viosterol daily. The earlier radiographs indicate that rickets was absent in the radii and ulnae, and the later films show that treatment with 15 drops of viosterol daily for one year produced dense terminal zones even in the bones of a *colored* premature infant. The absence of radiographic signs of rickets, even in the patient receiving but 10 drops of viosterol daily, demonstrates the protective value of viosterol against the development of rickets in prematurely-born infants.

It is interesting that in the cases which came to autopsy the incidence of rickets in premature infants who received the usual amounts of cod liver oil during life was as great as in the group which did not

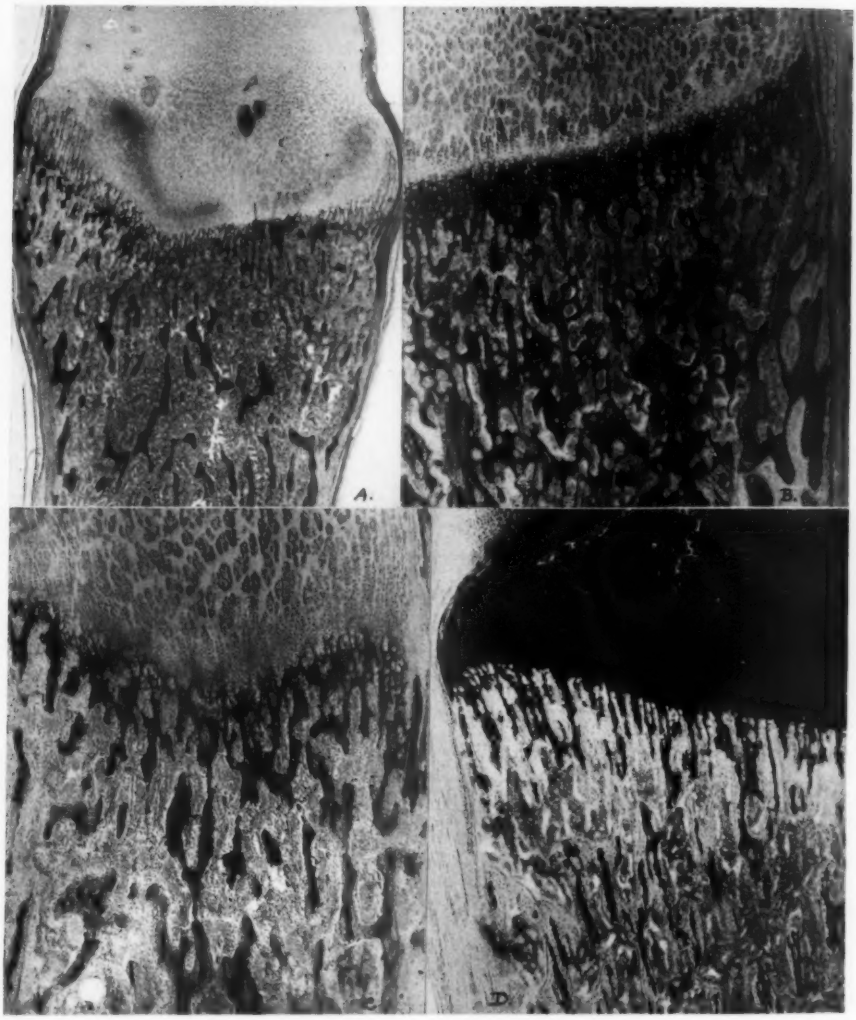

FIG. 5. MICROPHOTOGRAPHS OF RIBS FROM PREMATURELY-BORN INFANTS RECEIVING 10 TO 20 DROPS OF VIOSTEROL DAILY FOR FROM ONE TO ELEVEN MONTHS

A, from a two-month-old infant who received 5 to 10 drops for fourteen days and 15 drops of viosterol for thirty-nine days, or for a total of fifty days; *B*, from a ten-week-old colored female who received 15 drops of viosterol for thirty-three days; *C*, from a four-month-old colored male who received 20 drops of viosterol for six weeks and 10 drops for nine weeks; *D*, from an eleven-month-old colored female who received 20 drops of viosterol, beginning with the first week of life. Note the absence of any signs of rickets in all four cases, especially in the younger infants during the period of most rapid growth—the first three months of post-uterine life.

receive any vitamin D at all. The incidence was 50 per cent (3 out of 6) in the first group and 40 per cent (4 out of 10) in the second group.

FIG. 6

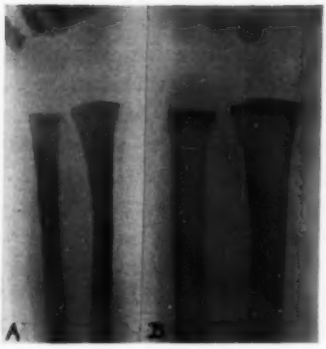

FIG. 7

FIG. 6. X-RAY PICTURES OF THE ARMS OF A PREMATURELY-BORN COLORED INFANT WHO RECEIVED 10 DROPS OF VIOSTEROL DAILY FROM THE FIRST WEEK OF HER LIFE UNTIL THE AGE OF ONE YEAR

A, X-ray film taken after two months; and *B*, after one year of therapy. Note absence of rickets at the distal ends of these bones as well as at the proximal ends of the ulnae.

FIG. 7. NORMALLY CALCIFIED ENDS OF RADIUS AND ULNA OF A FOURTEEN-MONTH-OLD COLORED PREMATURELY-BORN MALE WHO RECEIVED 15 DROPS OF VIOSTEROL DAILY, BEGINNING AT SIX WEEKS OF AGE

A, taken after ten weeks; and *B*, after one year of therapy. Note the dense terminal zones, indicating superior ossification, in *B*.

Compared with viosterol, the efficacy of cod liver oil, in ordinary doses as a prophylactic agent against rickets in this group of premature infants, appears to be very limited.

From our clinical observations and pathologic study, the following points in the prevention of rickets in the premature suggest themselves:

(a) That larger doses of vitamin D, such as 20 drops of viosterol, should be given daily soon after birth and continued for a period of four or five months—the period of most rapid growth. The dosage of viosterol may then be reduced to the level for full-term infants, namely, 5 to 10 drops daily.

(b) That in spite of adequate amounts of viosterol, the bones, although not rachitic, may show varying degrees of osteoporosis, due, in all probability, to an actual paucity in the intake of calcium phosphate. Accordingly, if a premature infant is gaining in weight at a very rapid rate, it may be advisable to supplement the diet with calcium phosphate.

The results of viosterol in the prevention of rickets in twins and syphilitic infants were the same as in prematures. In fact, a number of the infants in this group have already been included among the prematures. There were 9 pairs of colored and 17 pairs of white twins, or a total of 52 individual infants. Of the congenital luetics, 8 were colored and 3 were white. Of the entire group of 63, 4 received 5 drops of viosterol, 28 received 10 drops, 13 received 15 drops, 17 received 20 drops, and but 1 luetic colored infant received 30 drops of viosterol daily. None of these showed evidences of rickets clinically or roentgenologically.

9. THE THERAPEUTIC EFFECTS OF VIOSTEROL IN ACTIVE RICKETS

As mentioned previously, only cases showing demonstrable clinical and x-ray evidence of active rickets in which it was possible to follow the healing process to completion were included in this group. There were 189 in all: 179 colored and 10 white. Of this number 25 received emulsions of viosterol instead of viosterol in oil. The therapeutic results with the emulsions in active rickets will be discussed in a separate communication. The age distribution of the entire group, when rickets was first diagnosed, is shown in figure 8. It is seen that the greatest incidence of rickets in our group occurred between the fourth and the fifteenth month of life, and declined quite sharply toward the second half of the second year. If a period of a month or

two is allowed for the development of the disease, the highest incidence of *beginning rickets* would probably fall between the third and twelfth months of life. This age incidence of rickets agrees quite well with the clinical observations of Eliot (7, 8) in New Haven, and with the necropsy findings of Schmorl (25) in Dresden and of Park, Eliot and Jackson (26) in Baltimore and New Haven. Eliot made roentgen examinations of the lower ends of the radius and the ulna of a group of 1,186 children, including both Negroes and Italians, and found that rickets began to be common at the end of the third month, reached its maximum incidence of 47 per cent at the seventh month, and then

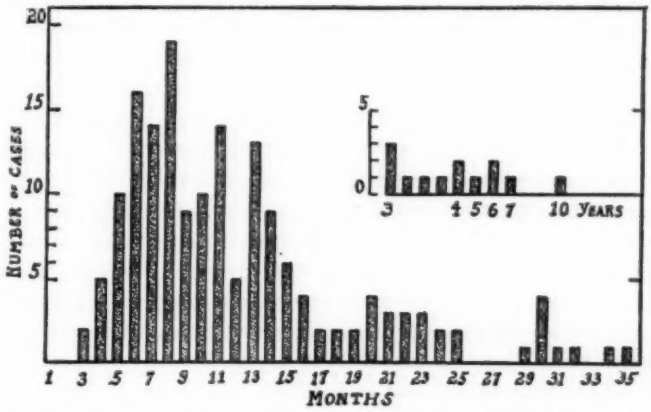

FIG. 8. SHOWING THE DISTRIBUTION OF RICKETS IN 189 CASES, ACCORDING TO AGES WHEN RICKETS WAS FIRST DIAGNOSED

declined in frequency, reaching about 18 per cent at the end of the second year and 4 per cent at the end of the third year. Schmorl (25) and Park, Eliot and Jackson (26) found a similar age incidence of rickets in several hundred autopsies and called attention to the infrequency of active rickets after the second year of life. These clinical and pathologic studies, therefore, suggest that the demarcation point between infantile and "late" rickets may be placed arbitrarily about the end of the second year. Accordingly, we have separated the cases of rickets occurring in the first two years of life—infantile rickets—from those in which the disease was first recognized after that period.

The latter group was designated as "late rickets" and comprises 23 patients ranging from 2 to 10 years of age. A report of the effect of viosterol in this group appeared elsewhere (27).

In a previous publication (27), we have called attention to the prevalence of active rickets in severe form in the colored as compared with the white race. Thus, of 23 children suffering from "late rickets," 21 were colored and but 2 were white; and of the latter, one had celiac disease, also. The prevalence of rickets among colored children becomes even more striking when it is seen that in our present group, comprising 189 cases in all, only 10 were white; and of the latter, 3 were premature, one suffered from celiac disease, one was a rapidly growing Italian child, and the remainder had but mild rickets. Although, a great preponderance of colored over white children was expected in our group, the 19:1 ratio of colored to white children was surprising. In the Harriet Lane dispensary, through which all patients, including those of the hospital and special clinics, pass, the number of colored and white children is approximately equal. Tuberculosis is thought to be much commoner in the colored than in the white race, yet in our clinic for tuberculosis the numbers of colored and white children are approximately the same. Therefore, the large percentage of colored children in our rickets group is a striking illustration of the prevalence of rickets in severe form in the colored race. The greater incidence is probably due to the diet and the living conditions of the colored children in Baltimore which are, on the average, far inferior to those of the whites. Then, too, the Negro mother in Baltimore is, on the whole, much more indifferent and unobservant of the health of her children than is the white mother. In consequence, she is less liable to seek medical aid early and is less persistent and reliable in carrying out such medical treatments even when they have been made available. Undoubtedly, the dark skin, which filters out ultraviolet light, and possibly a racial inherited susceptibility are additional factors in causing the high incidence of rickets in the colored race. The rôle of ultraviolet light in the etiology of infantile rickets is illustrated in figure 9, which is based on the analysis of 160 cases, in which there was reason to believe that the disease was not of long standing. It shows unmistakably the prevalence of the disease in the winter as compared with the summer months and that the curve of the

incidence of rickets corresponds to the season when but small amounts of ultraviolet light are available in Baltimore, *i.e.*, during the fall and winter months (28). The seasonal incidence of rickets in our group closely resembles that found by Schmorl (26) in 368 consecutive necropsies on infants and children. His data, arranged according to percentages of florid and of healing rickets, indicate the prevalence of

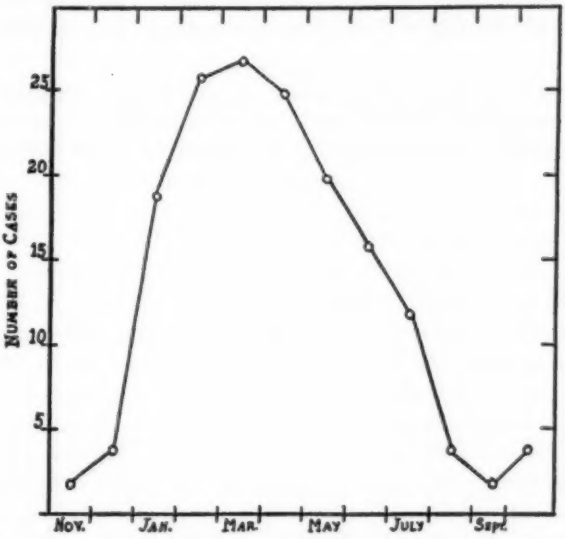

FIG. 9. SEASONAL DISTRIBUTION OF RICKETS IN 160 CASES IN WHICH THERE WAS REASON TO BELIEVE THAT THE RICKETS WAS NOT OF LONG-STANDING

florid rickets during the Winter months and the tendency to spontaneous healing during the summer.

The distribution of the severity of rickets in our entire group of 164 cases, at the time viosterol therapy was begun, was as follows: Mild, 8; moderate, 46; severe, 38; very severe, 72. Of the 8 children showing mild rickets, 3 were white; and of these, 2 were prematurely-born. In analyzing the results of viosterol in the cure of rickets, we have separated the 4 groups and attempted to determine the relation of the severity of the disease and of the dosage of viosterol to the time required to heal completely the rachitic process. This, however, was

not possible in all instances, owing to the fact that, in some cases, there was considerable lapse of time between the taking of the final x-ray films showing complete healing and those taken when healing was still in progress. Under these circumstances, the exact time it took to heal completely the rachitic process could only be roughly estimated. Indeed, as pointed out previously (27), even in cases in which x-ray films of the bone are taken at frequent intervals, the stages in the healing of rickets can be recognized in only the roughest

TABLE 6

Showing (1) distribution of 134 cases of rickets according to dosage of viosterol, (2) length of time required to cure the rickets, and (3) average healing-time on the various dosages

The numerals in each square indicate the number of cases in each group.

DROPS OF VIOSTEROL	MONTHS REQUIRED TO HEAL																				AVERAGE DURATION
	1		2		3		4		5		6		7		8		9		10		
																					months
10			1		2	2	3		2		2	1		1						1	5.0
15	1		1		2	1			1	2		1			1						4.3
20		1	6	3	8	1	3		3												3.1
30			6	3	9	2	2	1		3			1								3.3
40		2	2	5	9	6	4	2		2	1	1	1		1						3.6
50					1	2		1													3.8
60		1	3		3	2	2		3				2	1							4.0
Totals....	1	4	19	11	34	16	14	4	9	7	3	3	4	2	2					1	

Per cent of cases healed within 5 months = 83.4.
Average healing-time for this group = 3.1 months.
Average healing-time for the entire group = 3.7 months.

way, since it is practically impossible to catch the process at exactly the right moment. We were able, however, to collect 134 out of the 164 cases, in which the length of time required for complete healing with viosterol could be reasonably evaluated, and these are represented in table 6 and in figure 10. Table 6 shows: (1) that in 83.4 per cent of the patients the rickets healed within five months; (2) that the average healing-time for the entire group, which includes cases of very severe slowly-healing rickets, was 3.7 months; and (3) that the average healing-time in the 83.4 per cent of the cases was

3.1 months. Figure 10 shows the relation between the ages of the patients at the time viosterol therapy was begun, and the relation of dosage of viosterol to the time required to heal completely the various degrees of rickets as determined by x-ray examination. It is seen that the severity of the rachitic process is of greater importance in this time-relationship than are either the ages of the patients in this group or the dosages of viosterol employed. Thus, in the mild and moderately severe cases of infantile rickets, complete healing occurred between one and one-half and three months; whereas, in the severe and the very severe cases, complete healing usually took place only after three to five months. The variation in the healing-time of rickets of the severe type is due, in all probability, to: (1) differences in severity of rickets which are not readily detectable by the x-ray; (2) differences in the environment and exposure to sunlight even in the winter months; (3) variations in diet and hence in the intake of calcium and phosphorus; and (4) possibly also the differences in the regularity with which the viosterol had been administered, although, as far as we could determine, the dosages and directions for its administration had been followed quite well. It is also possible that the presence or absence of infection may have been a factor in the variability of response of our patients to viosterol, since Töpfer (29) has recently shown that infections definitely retard the effects of vitamin D in rachitic infants. We realized that, had all our patients with rickets been hospitalized and their diets and intake of vitamin D carefully supervised, this variation would have been minimized to some extent. But, on the other hand, this is exactly what we tried to avoid. After all, the aim in the attack on rickets is not to hospitalize rachitic children and give them the benefits of hospital food and care, as is frequently done with small groups under investigation, but to make antirachitic therapy available at a minimum of expenditure to all those afflicted with the disease and obliged to live under the conditions prevailing at their homes.

There is no doubt that variability in diet is responsible to a large extent for the differences in the length of time necessary to bring about complete healing of severe rickets. The economic status of the negro families in Baltimore is far inferior to that of the white, so that the diets of colored children are correspondingly inadequate and im-

properly balanced. For example, until very recently, when the relief agencies began to provide milk for families with small children, this article of diet was not available to many children between the ages of nine months and two years and they were thus forced to subsist on diets low in calcium phosphate. We have discovered that in some of our patients the slowness of healing was actually due to such a dietary deficiency, and when this was corrected by providing the family with milk and vegetables, the rickets healed more rapidly. In cases of very severe rickets where such provision was not possible, the deficiency was rectified by the addition of 1 to 3 grams of calcium phosphate daily. These procedures, however, were carried out only in a limited number of cases, so that in most of the other cases of severe rickets there existed not only differences in degree of severity of the disease, but also variations in the amounts of lime salts ingested daily.

Notwithstanding the fact that in some of the cases of florid rickets the healing-time was considerably prolonged, others healed within two to five months, the average healing-time for the entire group being 3.7 months. Compared with the efficacy of cod liver oil, the results with viosterol are indeed satisfactory. In the average case of infantile rickets of moderate severity, with the patient under treatment with 3 teaspoonfuls of potent cod liver oil daily, the deposition of lime salts at the cartilage-shaft junctions appears within about twenty-one days; the ends of the bones seem to become nearly fully impregnated with lime salts at about the end of two months; and the ends of the bones approximate a normal definition and appearance after five months. In the severe and the very severe types of rickets, however, this amount of cod liver oil may fail entirely to cure the disease or it may heal it only partially, so that the defective calcification may still be evident after a year of cod liver oil administration. Larger amounts of the oil are usually not tolerated by most children so that with the ordinary dose the disease frequently "smolders" on and leads to the formation of extreme deformities of the chest and extremities. In the average case of rickets treated with moderately large doses of viosterol, deposits of lime salts may appear at the cartilage-shaft junctions in as short a time as a week or ten days; and if the rickets has been of short duration and hence of moderate degree, so that the

rachitic zones at the ends of the shafts are relatively narrow, the ends of the shafts may be completely ossified and present an approximately normal appearance within three months or even sooner. In the severer types of the disease, the healing period with viosterol therapy may sometimes be prolonged, but ultimately at least it effects a cure and eradicates the rachitic process completely.

In figure 10 there appears to be very little difference in the healing-time in relation to dosage, as far as the cases presenting mild or mod-

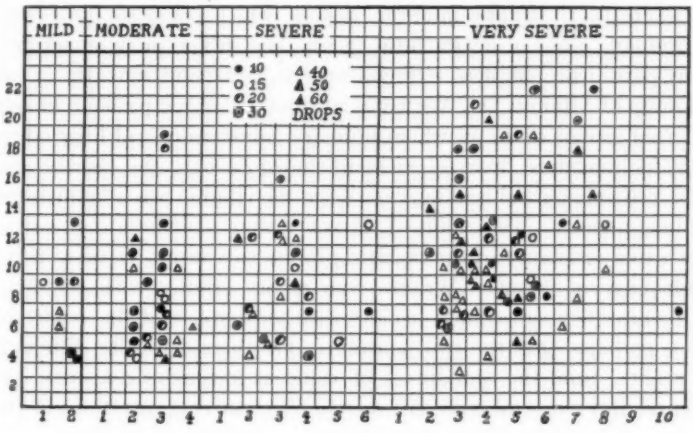

FIG. 10. RELATION OF DOSAGE OF VIOSTEROL (IN DROPS, DAILY) AND AGE OF PATIENTS TO THE TIME REQUIRED TO HEAL COMPLETELY RICKETS OF VARYING SEVERITY. (ONE HUNDRED AND THIRTY-FOUR CASES)

Abscissae, time in months; ordinate, age in months

erate rickets are concerned, but in the severe and very severe case, the use of larger doses of viosterol seems to have shortened the period of healing as compared with that brought about by smaller dosages. Thus, in the former groups, the rachitic process healed nearly as rapidly with 10 or 15 drops as with 50 or 60 drops of viosterol daily; whereas, in the latter groups, the average periods of five and six months required to heal severe and very severe rickets, respectively, with 10 to 15 drops of viosterol daily could be shortened to three or four and

a half months by using twice these dosages. From this study, it may be surmised, therefore, that 20 to 30 drops of viosterol daily will produce a degree of healing in very severe cases of rickets equal to that accomplished by larger doses; and that unless there is some indication for using larger amounts, as for example, when there is reason to believe that vitamin D is poorly absorbed or excreted before adequate absorption takes place, there is no reason for giving extremely large doses of viosterol, even in the most florid cases of infantile rickets.

The effects of viosterol in "late" rickets are discussed more fully elsewhere (27). Unlike infantile rickets, which responds quite well to ordinary doses of antirachitic agents, late rickets may sometimes show partial or complete refractoriness to treatment with the usual amounts of vitamin D. We have encountered some patients in our reported groups of 23, in whom ordinary amounts of cod liver oil or small or moderate doses of viosterol failed to cure the disease and produced only partial healing, even when the vitamin was administered for long periods. These failures, however, may have been due, not so much to a resistance of the rachitic process to vitamin D *per se* as to inadequacy of dosage. Writers who report failures with cod liver oil therapy but subsequent healing with viosterol, lead the reader to infer, perhaps, that the two substances are therapeutically different. In reality, from the standpoint of value as antirachitic agents, cod liver oil and viosterol represent merely different concentrations of the same or of a similar therapeutic agent[9]—vitamin D. Cod liver oil or equivalent amounts of viosterol produce an effect in late rickets, but the effect may be so small as not to be demonstrable by the ordinary methods of examination. To understand the relationship of the dosage of cod liver oil and viosterol to healing of the rachitic process, one must grasp two facts: (1) that there are all degrees of rickets, for example, florid rickets with no lime deposition at the rapidly growing ends of the long bones, and rickets with 10, 30, 50 or 98 per cent of the normal deposition of lime salts, as the case may be; and (2) that these degrees of rickets may continue in the same state

[9] Therapeutic identity does not necessarily mean chemical identity. It is conceivable that there are several chemically different or isomeric forms of vitamin D and that they may have the same therapeutic effect in the prevention and cure of rickets.

of partial activity for weeks, months or years. When the rachitic tendency is especially great, either from extrinsic or intrinsic causes or both, an ordinary dose of cod liver oil or of an equivalent amount of vitamin D in viosterol merely raises the healing process to a somewhat higher level but not to a state of active healing. With larger doses of vitamin D, such as can be given in viosterol, the healing tendency may be accelerated so that the rachitic process may be brought to a termination in a relatively short time. Thus, the great value of viosterol in severe rickets lies in its concentration in vitamin D (and, to a lesser extent, in its ease of administration) and in its corresponding ability to cure rickets when other antirachitic agents prove inadequate.

Generally speaking, most of our patients with late rickets responded quite well to viosterol therapy, especially those who received from 40 to 60 drops daily. In some instances, the relatively long period required for complete healing could be traced to inadequacy of vitamin D or to a paucity of lime salt in the dietary, but in some of the older patients, as for example, the seven-year-old colored boy, whose x-ray films of the lower extremities are shown in figures 15 and 16, the rachitic process healed completely only after about eight months of therapy with 60 drops of viosterol daily. In such a case, however, a more rapid progress could hardly be expected, especially when one considers the amount of osteoid tissue present in the metaphyses of the long bones and the severity of the osteoporosis of the entire skeleton in this case of chronic rickets. It is remarkable, indeed, that such a degree of impregnation of the skeleton with lime salts took place even in this relatively long period, for in cases of osteitis fibrosa of the osteoporotic type, the same degree of recalcification does not occur even within a year after parathyroidectomy has been performed and an abundant supply of lime-containing food has been provided.

Only in one case, that of a white girl of ten years, did we encounter the possibility of failure with viosterol. In this instance, however, the test was not a fair one, since the dosage of viosterol was relatively small—20 drops daily—and the period of administration was only forty-five days, during which time she was also exposed to irradiation from the mercury quartz lamp. But, even in this case, there was some healing demonstrable in the x-ray films; and had this patient continued to take a larger or even the same dosage of viosterol for longer periods,

the rickets probably would have healed completely, as it did in the other patients with late rickets.

With the exception of the instance just cited, we have not met with a single case of rickets which was entirely resistant to viosterol therapy. Others, however, have encountered such refractoriness, not only to cod liver oil, but also to viosterol. The resistance of celiac disease to cod liver oil has been stressed by Parsons (30); and Blackfan (31), Bakwin (32), McCune (33), Stearns, Oelke, and Boyd (34), Jampolis and Londe (35), and others have reported a similar resistance in what appears to be "ordinary" rickets. We, too, have seen a case of ordinary rickets in a white girl of four years in which the disease was totally refractory to cod liver oil therapy and treatment with ultra-violet rays from the mercury quartz lamp, although carried on for a few months. We had no opportunity to try the effectiveness of large doses of viosterol in this patient, so that we are unable to say how this powerful antirachitic agent would have affected the rachitic process in this instance. Even in some of the cases just cited, the resistance to vitamin D was only partial, since the rickets could be ultimately influenced by larger doses of viosterol. In McCune's patient, a definite retention of calcium and phosphorus was obtained by the administration of a special preparation of viosterol, containing about 100 times as many rat units as ordinary commercial viosterol; and in Jampolis and Londe's patient the rickets healed completely after the administration of very large doses of a similar preparation.

What makes some types of rickets more resistant than others to vitamin D therapy is difficult to say. It has been suggested that there might be an actual defective absorption of the vitamin from the gastrointestinal canal, as is probably the situation in celiac disease and in sprue. This, however, could hardly be the case in ordinary rickets without concomitant disturbances in intestinal function. First, the rickets in refractory patients may be as resistant to ultra-violet light as to oral medication; and secondly, vitamin D may be demonstrated in the blood of such patients shortly after the ingestion of viosterol (33), thus indicating no gross interference with absorption from the gastrointestinal canal. The activity of the parathyroids, which are often found to be enlarged and probably hyperfunctioning in rickets of long standing, may also play a rôle in preventing the

antirachitic activity of vitamin D, but exact knowledge on this point is still lacking. The failure of rickets to respond to antirachitic treatment may occasionally be due to the overzealous addition of calcium salts, other than calcium phosphate, to the diet. Physicians are often led to believe that adding soluble calcium salts to the dietary of the patient results in better absorption of calcium and hence in improved calcification. However, the invalidity of this notion can be readily demonstrated experimentally in animals and also in human beings. Indeed, in animals, if the dietary phosphorus is low, the rickets-producing properties of the diet are greatly enhanced by increasing the calcium, and the antirachitic effect of vitamin D can be increased by decreasing the calcium or, far better, by increasing the phosphorus intake. Conversely, the antirachitic activity may be diminished by decreasing the phosphorus or increasing the calcium intake. Schabad (36), Bosworth and Bowditch (37), and Orr, Holt, Wilkins, and Boone (38) noted a similar effect on the retention of lime salts in children when either calcium or phosphorus was added to the diet. Thus, when Orr and his associates administered calcium chloride or calcium acetate to infants suffering from the low-phosphorus type of rickets, there occurred an added loss of phosphorus by way of the feces and a definite diminution of the concentration of inorganic phosphorus in the serum. We have noted a similar effect of calcium chloride on the healing of rickets by viosterol in the course of the administration of these substances for the cure of associated tetany. Ordinarily, moderate doses of viosterol produce a healing effect in the metaphyses of the bones of rachitic children within a period of ten to twenty days; whereas, when we added calcium chloride, the same amount of viosterol did not produce deposition of lime salts even after a month of therapy. On the other hand, we were able to effect more rapid healing in rachitic infants by the supplemental feeding of sodium phosphate, dicalcium phosphate, or of foods rich in phosphorus. It is thus possible that the resistance of protracted rickets to vitamin D therapy may have been due, in part, to an overfeeding with soluble calcium salts, as has been done in some of these cases, rather than to some intrinsic factor alone.

In considering refractory forms of rickets, one must rule out the existence of Morquio's disease (osteochondrodystrophy deformans).

The outer characteristics and even the x-ray films of the long bones of patients afflicted with Morquio's disease may resemble rickets to such a degree that it may actually be mistaken for the latter disease. We have seen three such patients who were treated for rickets for some time and apparently without benefit; and only after careful studies of the x-ray films of the bones did the nature of these osseous lesions become apparent. Indeed, most of the cases of Morquio's disease reported in the literature have been first considered as rickets rather than a dyschondroplasia. Anatomically and etiologically, the two maladies are, of course, quite distinct. Osteochondrodystrophy is a developmental defect in the orderly growth of cartilage and is merely a subdivision of achondroplasia (39).

10. EFFECT OF VIOSTEROL IN THE TREATMENT OF THE COMPLICATIONS OF RICKETS, AND THE VALUE OF EFFECTING RAPID HEALING OF RICKETS

Rachitic deformities. Rickets *per se* is seldom fatal. If the disease process in the skeleton has not progressed to a florid state and therapy is instituted promptly and adequately, local or remote sequelae are indeed rare. If, however, the disease has passed into and has remained in a state of severity for some time, it may then leave its imprint on the skeleton in the form of deformities of the long bones, chest, head, and pelvis. The most common sites for deformities to occur are the lower extremities and pelvis, owing to the fact that the soft rachitic bones yield to the force applied to them in sitting, standing, or walking. In order to forestall the development of deformities, it is important to prevent pressure on the extremities and also to increase the rigidity of the bones to a point where the weight of the body will be supported without yielding. Cod liver oil in ordinary dosages, or small amounts of viosterol, accomplish the hardening process very slowly and inefficiently, so that, in spite of the ultimate healing of the rachitic process, there usually remain deformities, especially of the femora, tibiae, and fibulae. Larger doses of viosterol, however, may accelerate deposition of lime salts in the soft and yielding skeleton and thereby avert deformities. An example of this is shown in figure 11, the x-ray films of the lower extremity of a fifteen-month-old colored girl suffering from a most severe type of rickets, complicated by multiple fractures and deformity of the thorax with

atelectasis (see also figs. 1–D and 19). Therapy with 60 drops of viosterol daily caused healing to advance so rapidly in the rachitic

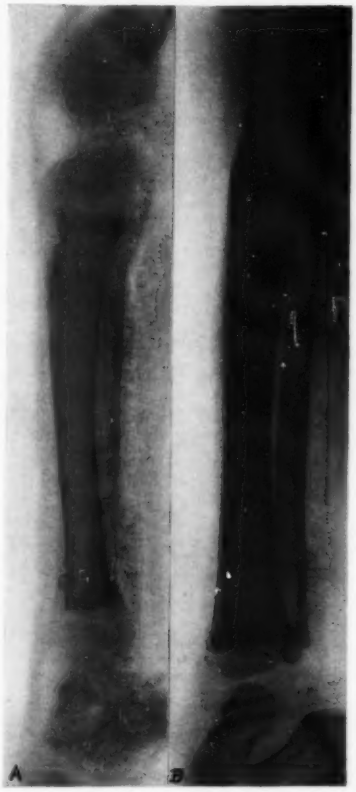

FIG. 11. ILLUSTRATING ABSENCE OF RACHITIC DEFORMITIES OF THE TIBIA AND FIBULA IN A COLORED GIRL OF FOURTEEN MONTHS, WITH VERY SEVERE RICKETS, TREATED WITH 60 DROPS OF VIOSTEROL DAILY. *A*, BEFORE THERAPY; *B*, AFTER THERAPY

(From same patient whose bones are shown also in figures 1–D and 21)

metaphyses and at the sites of the various fractures that the bones became rigid enough to withstand the weight of the body without

FIG. 12. DEVELOPMENT OF SABRE-SHIN DEFORMITY IN A COLORED BOY OF TWO YEARS, RECEIVING 10 DROPS OF VIOSTEROL DAILY FOR TWO AND ONE-HALF MONTHS AND 15 DROPS DAILY FOR AN ADDITIONAL SIX MONTHS

The child suffered from mediastinal tuberculosis and subsisted on a very poor diet. He weighed only 24 pounds at three years of age. Although his parents claimed that he received cod liver oil regularly for seventeen months, he presented severe rickets with fractures of the bones at the age of two years. The slowness of healing with a moderate dose of viosterol, the poor diet, and postural forces in standing or in walking, in a patient of this age, probably contributed to the occurrence of the deformity; *A*, before therapy; *B*, after six months; and *C*, fourteen months after viosterol therapy was begun.

yielding. In other instances in which the rickets was not of such severity as in the above case, deformities of the lower extremities developed, though slow healing was in progress. This contrast is illustrated in figure 12, the x-ray picture of the legs of a colored boy of two years, who received 10 to 15 drops of viosterol daily, and in whom the rickets healed completely only after eight and one-half months.

If deformities of the lower extremities exist before vitamin D therapy is begun, their successful correction by osteotomy or osteoclasis depends, to a large extent, also on the rate of healing. If the healing is slow, there is always the possibility that the fractures will also heal slowly and this will allow a new deformity to occur at the sites of operation; but if healing can be accomplished rapidly, as when the diet is made adequate and fairly large doses of vitamin D are administered, the recurrence of deformities may be entirely averted. *A priori*, it would seem that the logical time to correct rachitic deformities of the lower extremities is while the bones are still soft and pliable, but experience has proved that this is not always the correct procedure to follow. We have found that the chances of a successful correction are better if osteotomy is performed when the healing of the rachitic process is far advanced rather than when the rickets is quite active and there is little evidence of healing. If, perchance, the diet is inadequate with respect to calcium phosphate and the dose of vitamin D is small, the possibility of the appearance of new deformities at the site of operation is, of course, increased. The contrast between the effects of slow and rapid healing on the correction of deformities of the lower extremities is illustrated in the following three cases.

The first case is that of a three-year-old colored boy with extreme rickets in whom osteoclasis of the tibiae and fibulae was performed one month after the daily administration of 20 drops of viosterol was begun. Although this dose of viosterol produced, within eighteen days, a sharp healing reaction at the cartilage-shaft junction in the form of heavy deposits of lime salts, it later proved to be insufficient to bring about complete healing, for during the eight months of its administration the disease process remained in the same state of partial development, the rachitic and the healing processes going on simultaneously. The poor response to therapy was also evident in the low

calcium concentration in the serum—8.6 mg. per cent. On investigation, it was learned that the child's diet was inadequate and not well

FIG. 13. EFFECT OF VIOSTEROL AND THE CONTINUED DEVELOPMENT OF THE SABER-SHIN DEFORMITY IN A COLORED BOY THREE YEARS OLD SHOWING EXTREME RICKETS

A, shows the tibia one month before the osteoclasis was performed for correction of the saber-shin and the viosterol therapy begun. Note the extreme degree of rickets without any signs of healing. *B*, shows the tibia shortly after the osteoclasis and after three months of therapy with viosterol. *C*, was taken eleven months after *A*, nine and one-half months after the operation and eight months after the treatment with 20 drops daily of viosterol was begun. The ends of the bones show the combination of healing and active rickets. The twenty drops of viosterol have checked the rickets but have been unable to bring about the final healing. Note, also, that the saber-shin deformity has increased. *D* was taken five months after C, and four and one-third months after the daily administration of 60 drops of viosterol. Note that the healing is essentially complete, and that the saber-shin deformity has progressed.

balanced, thus accounting for the slow healing. When the diet was fortified with milk and vegetables and the dosage of viosterol in-

creased to 60 drops daily, there was a rapid rise in the concentration of calcium in the serum to normal values and an increase in the deposition of lime salts in the osseous tissue. But, as far as the deformities were concerned, this later procedure was of no avail. They were actually worse than initially. The sequence of events is illustrated

FIG. 14. EFFECT OF "LATE" OSTEOTOMY ON THE CORRECTION OF RACHITIC DEFORMITIES. L. O., COLORED FEMALE, AGE TWENTY-SEVEN MONTHS

A, before viosterol therapy. Note the extreme rickets and the backward displacement of the lower epiphysis of the tibia. *B*, after therapy with 20 and 60 drops of viosterol daily for three and four weeks, respectively. *C*, after four months of therapy and shortly before osteotomy was performed. *D*, six months after osteotomy. Note that the correction was successful and that the site of operation healed completely under viosterol therapy.

in figure 13: "A" shows the tibia one month before osteoclasis was performed for the correction of the saber shin and the viosterol therapy begun. "C" was taken eleven months after "A," nine and one-half months after the osteoclasis was done, and after eight months treatment with 20 drops and one month with 30 drops of viosterol daily.

"D" was taken five months after "C," and four and one-third months after the administration of 60 drops of viosterol daily was begun.

The second case is that of a colored girl of two years and seven months, whose rickets and deformities of the lower extremities and chest were much worse than those of the preceding patient, but in whom the rachitic process healed quite rapidly on 60 drops of viosterol daily—within a period of eleven weeks. In her case, osteotomy was

FIG. 15. ROENTGENOGRAMS OF THE UPPER EXTREMITIES OF A COLORED BOY, AGED SEVEN YEARS, WITH EXTREME RICKETS AND OSTEOPOROSIS, CURED WITH 60 DROPS OF VIOSTEROL DAILY

A, before treatment; *B*, after two and one-half months of therapy; and *C*, after eight and one-half months of treatment with viosterol. The effect of viosterol on the healing of the rickets in the lower extremities is shown in Figure 16.

performed after viosterol had been administered for about four months; it was continued for an additional three months after osteotomy. The fracture healed quite rapidly, and a successful correction was maintained (fig. 14).

The third case is that of a colored boy of seven years with extreme "late" rickets and osteoporosis, who had been able to get around only by crawling. The x-ray appearances of the upper extremities are

illustrated in figure 15, and those of the lower extremities are shown in figure 16. Therapy with 60 drops of viosterol daily produced a slow but progressive healing in this markedly rachitic child, so that at the end of seven months of treatment the bones became sufficiently rigid and osteotomy was performed. The fractures healed rapidly with good formation of callus, and in this way the deformities were suc-

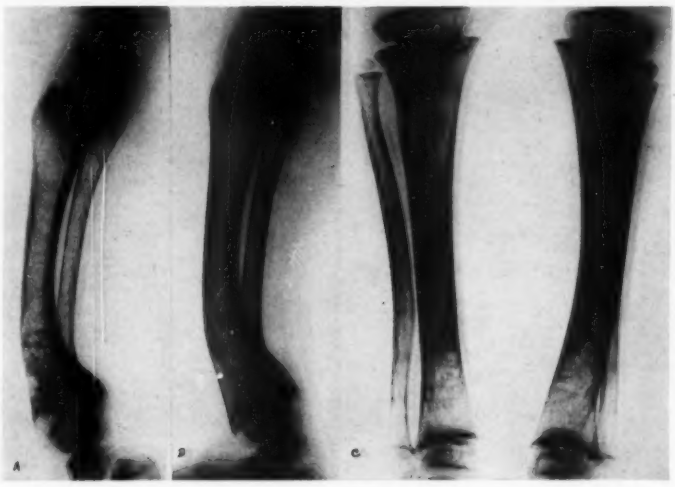

FIG. 16. EFFECT OF VIOSTEROL AND OF "LATE" OSTEOTOMY ON THE RICKETS AND ON THE CORRECTION OF THE DEFORMITIES OF THE LOWER EXTREMITIES, IN THE PATIENT SHOWN IN FIGURES 15 AND 17

A, before therapy; *B*, after four and one-half months of therapy with 60 drops of viosterol daily; and *C*, four months after osteotomy was performed, and eleven months after viosterol therapy was begun. The result of the treatment and osteotomy is shown more strikingly in Figure 17.

cessfully corrected. From this time, the boy began to walk normally and gained in weight and height. The result of viosterol therapy and of osteotomy on his deformities and stature are shown in figures 15, 16 and 17.

If, after osteotomy, we have discovered that callus ossification is slow and the bones begin to yield, we supplement the diet with milk

or with 3 to 5 grams of calcium phosphate, and refer the patients to the orthopedist for the application of braces or plaster-of-Paris casts.

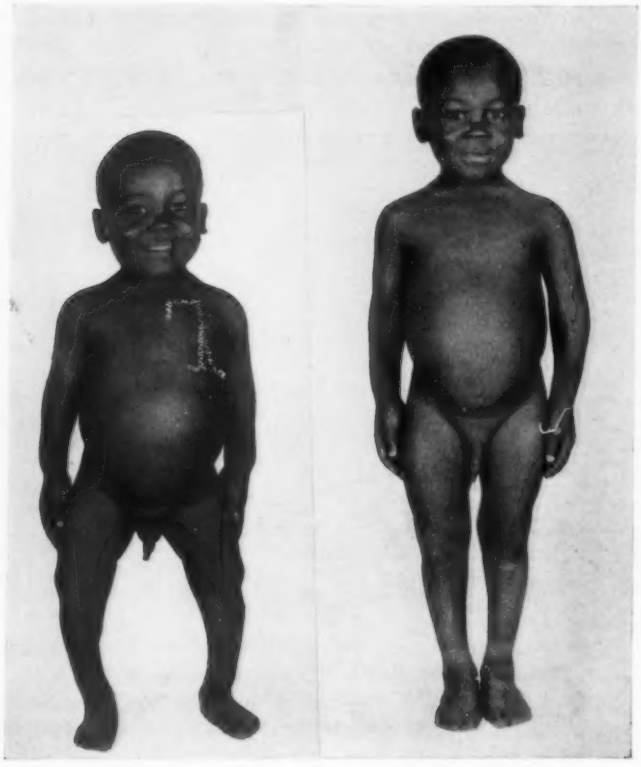

FIG. 17. SAME PATIENT WHOSE BONES ARE SHOWN IN FIGURES 15 AND 16. PHOTOGRAPHS TAKEN BEFORE AND AFTER EIGHT MONTHS' VIOSTEROL THERAPY; AND BEFORE AND SHORTLY AFTER OSTEOTOMY FOR THE CORRECTION OF THE DEFORMITIES OF THE LEGS

The gain in height was 8.5 cm.

In nine patients with severe, multiple, rachitic deformities of the lower extremities, instead of resorting to several operations, Dr. I. W. Nachlas and one of us (D. H. S.) have recently developed a non-

operative method for the correction of such deformities. Essentially, the method consists of feeding to such patients a diet low in phosphorus to which are added about 4.5 grams of strontium carbonate ($SrCO_3$) daily, until the bones become sufficiently pliable to be bent manually into the desired position, after which plaster-of-Paris casts are applied and the bones recalcified. For the purpose of recalcification, the strontium carbonate is discontinued and the diets changed to one rich in calcium and phosphorus with additions of moderate doses of viosterol—from 30 to 40 drops daily. The details of this procedure will appear shortly in another publication.

Thoracic rickets. The value of effecting rapid healing is also of inportance in cases of severe "thoracic rickets." In these very severe types of rickets, there is not only deformity but also interference with the physiologic functions of the thorax, as pointed out by Park and Howland (40). They found that, in children under two years of age suffering from the combination of rickets and extreme malnutrition, the thorax is often weakened and lacks rigidity, so that it fails in its function as a bellows. Most children with this condition have respiratory infections, but the infections themselves are relatively mild; it is in the loss of rigidity of the thorax that the essential danger lies, predisposing the children, as it does, to atelectasis and pneumonia. For this reason, it is essential to change the thorax from a collapsible bellows into one more rigid, with the least possible delay. We have found that viosterol, in moderately large doses, accomplishes this adequately.

Although x-ray films of the chest were taken in a large number of our cases of active rickets, serial roentgenographic studies were possible in only 24 instances. In these, the effect of viosterol was studied with a view to determining the improvement in the atelectasis as well as in the calcification of the chest. Like the patients studied by Park and Howland, most of the children included in our group also suffered from extreme rickets and malnutrition. Roentgenograms of three such patients treated with viosterol are shown in figures 18, 19, and 20. The first case is that of an eleven-month-old colored male with most pronounced signs of rickets in nearly every bone in his body, including the scapulae and pelvic bones. The chest was much deformed and the x-ray films showed marked compression atelectasis

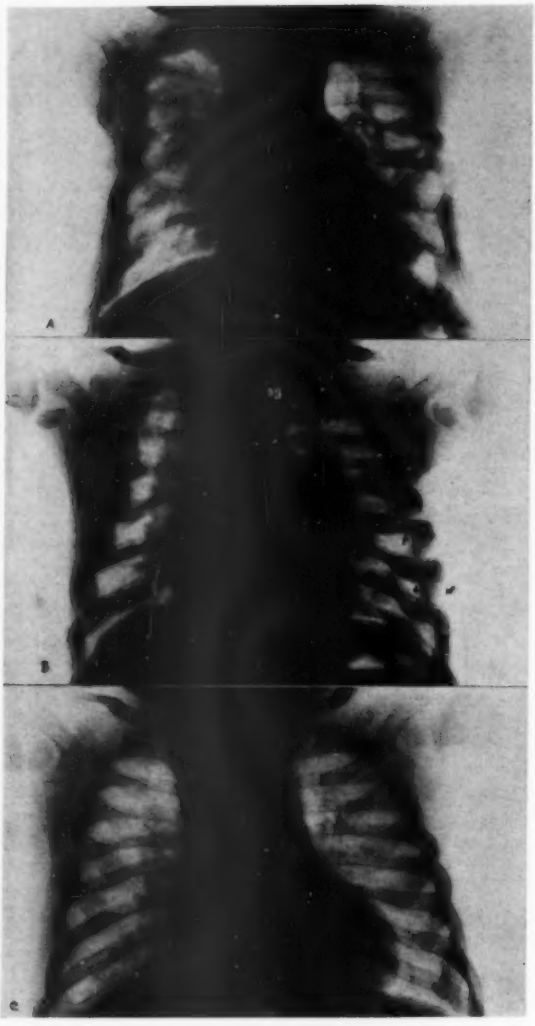

FIG. 18. EFFECT OF VIOSTEROL IN THE TREATMENT OF SEVERE THORACIC RICKETS IN A COLORED CHILD OF ELEVEN MONTHS

A, before therapy; *B*, after twenty days of therapy with 30 drops of viosterol daily and 3 grams $CaHPO_4$; *C*, after five months of treatment with viosterol. Note the atelectasis, the deformity of the chest, and the extreme porosity of the bones before therapy, and the improvement in ossification and in the lung fields after treatment with viosterol. Note also that the lime salt deposition in the ribs and scapulae (in B) is in much more advanced state than is usually brought about by viosterol alone, without the phosphate supplement.

(fig. 18–A). The second roentgenogram of the chest, taken twenty days after therapy with 30 drops of viosterol and 3 grams of dicalcium phosphate ($CaHPO_4$) daily had been begun, showed prompt and definite calcification in the ribs, clavicles and scapulae, and the increased density of the bones brought out the extent of the deformi-

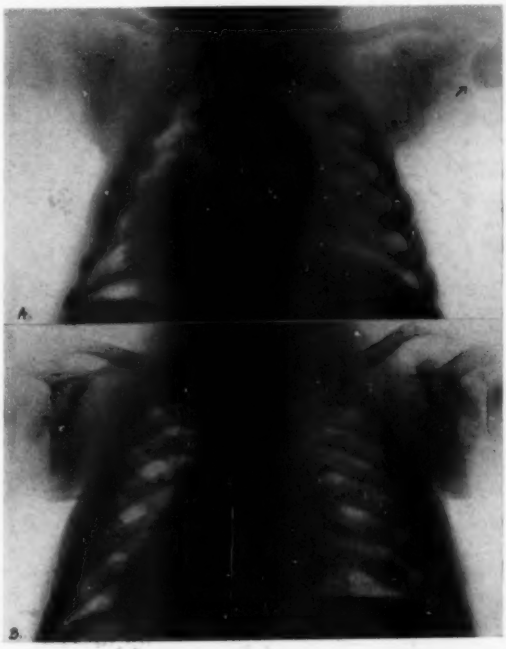

FIG. 19. IMPROVEMENT IN ATELECTASIS AND IN THE RICKETS OF THE CHEST IN A COLORED GIRL OF FOURTEEN MONTHS, TREATED WITH 60 DROPS OF VIOSTEROL DAILY

A, before therapy; *B*, after six months of treatment. (From same patient whose bones are shown in figures 1–D and 11.)

ties in the ribs (fig. 18–B). Subsequent roentgenograms showed marked improvement in the chest deformity as well as in the atelectasis; and in the films taken five months after the commencement of viosterol therapy, the chest appeared fairly normal in contour and the atelectasis had almost entirely disappeared.

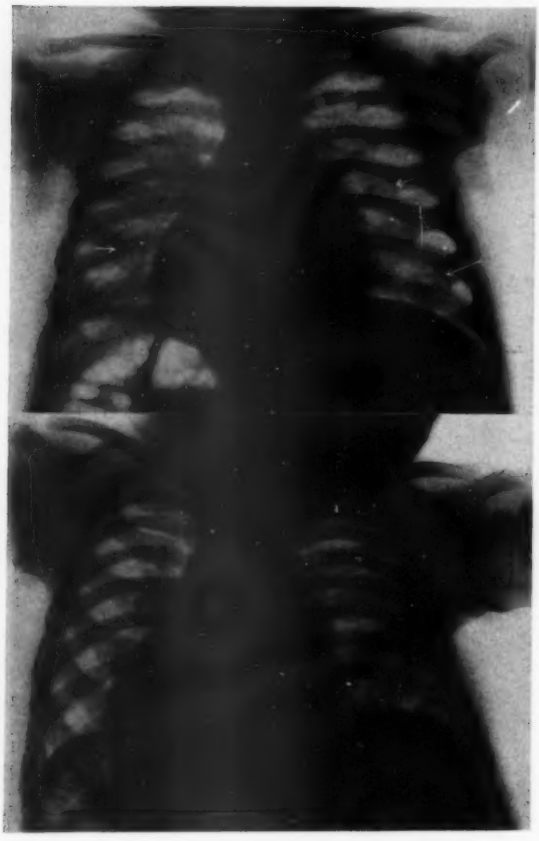

FIG. 20. EFFECT OF VIOSTEROL IN A CASE OF THORACIC RICKETS IN A COLORED CHILD OF TWENTY MONTHS, SHOWING MARKEDLY ENLARGED COSTOCHONDRAL JUNCTIONS

A, before therapy with 60 drops of viosterol daily; *B*, after one and one-half months of treatment.

The second patient, whose roentgenograms are shown in figure 19, presented a picture of extreme rickets with multiple fractures of many of the long bones (figs. 1–D and 11), a soft chest, and marked signs of

atelectasis. She was given 60 drops of viosterol daily and the rickets and pulmonary signs began to improve rapidly, so that by the end of a few months the thorax felt quite rigid, and the auscultatory signs of atelectasis had improved considerably. A comparison between an x-ray of the chest taken at the beginning of viosterol therapy and one taken six months later is made in figure 19.

A third example of the effect of viosterol on thoracic rickets is shown in figure 20. This twenty-month-old colored boy presented extreme rickets of the extremities and chest, as well as some auscultatory signs of atelectasis. The extent of the enlargement of the costochondral junctions may be seen from figure 20–A. Treatment with 60 drops of viosterol brought about improvement in the rickets so that, within one and one-half months, the chest assumed its normal rigidity and the beading of the ribs had diminished considerably (fig. 20–B).

Part II

11. Effect of viosterol in infantile tetany

The treatment of tetany in infants has undergone considerable change in the past two decades. There is no longer any question that most cases of infantile tetany result from some derangement in the metabolism of calcium and phosphorus, and that in the majority of these cases there is present also some evidence of rickets. With the advancement of knowledge concerning the treatment of rickets, the treatment of tetany has also been clarified; and like rickets, it too may be prevented by an adequate supply of vitamin D. It is, therefore, of interest that tetany did not occur in a single child in our entire prophylactic group. In the treatment of *active* tetany, vitamin D plays an important rôle. While the immediate symptoms of tetany can be alleviated by specific calcium therapy, the effect, although rapid, is but transient, the concentration of calcium in the serum usually receding to the previous tetany level within a few hours after the cessation of administration. Thus, when calcium is given without a true antirachitic agent, it may have to be continued, in doses of 3 grams or more daily, for as long as a month or even longer, lest the symptoms of tetany return (19). When, however, it is administered in conjunction with a powerful antirachitic agent, such

as viosterol, the rise in the concentration of calcium in the serum is sustained, the period of calcium administration is shortened considerably, and there is less likelihood that the symptoms will return. Unlike rickets, tetany is a serious symptom-complex requiring prompt attention; and hence it is the custom in our clinic to treat the immediate symptoms with calcium salts and then proceed with antirachitic agents. In most of our cases, therefore, vitamin D was not started at the first visit to the dispensary or immediately after admission to the wards, but some few days later. With these precautionary measures, patients with severe tetany were often given calcium salts before it was possible to obtain blood for calcium and inorganic phosphorus determinations, thereby reducing the number of cases in which the effects of therapy could be followed by chemical examinations of the blood. The number of cases so studied was further reduced by the failure, for one reason or another, to obtain blood for subsequent determinations, although the patients were observed clinically and the persistence or absence of symptoms of tetany was accurately recorded.

All in all, we were able to follow more than 100 cases of tetany which were treated with viosterol and calcium salts, but only in 25 of these were we able to follow the effects of therapy by frequent chemical examinations of the blood for calcium. The results in this group are shown graphically in figure 21. It is seen that, in most instances, the calcemia reached levels above those found in tetany in about seven to ten days after the beginning of vitamin D therapy. Clinically, the acute symptoms of tetany usually disappeared shortly after institution of vigorous calcium therapy, but the latent signs did not wane until some five to eight days after the combined calcium and vitamin D therapy was begun. It must be appreciated that not all patients with infantile tetany present identical or even similar symptoms and chemical findings in the blood, and hence there necessarily must exist considerable variations in the response to therapy. The variations in symptomatology are usually due to differences in the initial concentrations of calcium and inorganic phosphorus in the serum, the degree of rachitic involvement, and the presence or absence of infection. In patients with only moderate hypocalcemia or with the combination of *hypocalcemia* and *hypophosphatemia*, the alleviation of symptoms and the return of the serum calcium concentration

to non-tetanic levels can be accomplished with little difficulty; whereas, in infants with marked *hypocalcemia* or with a definite *hyperphosphatemia*, the return to a non-tetanic state can be brought about only by intensive and prolonged therapy. When the tetany is a com-

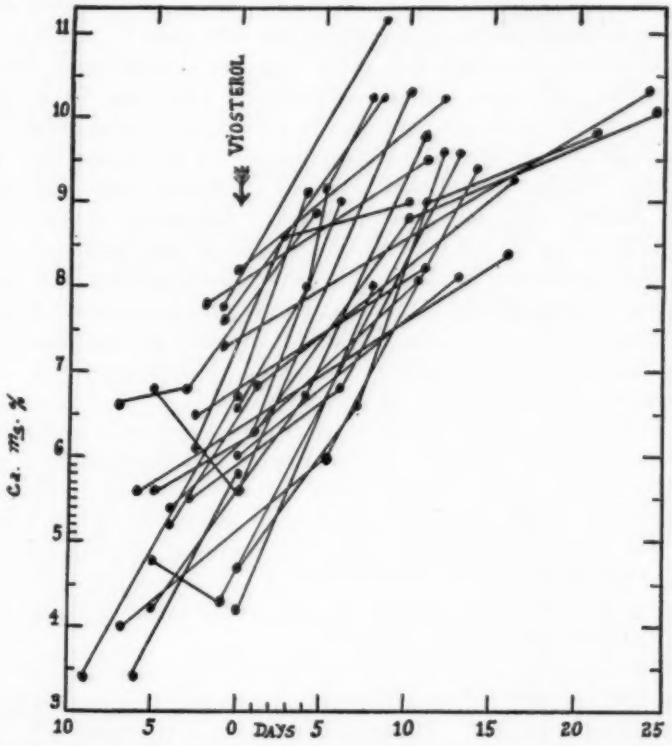

FIG. 21. EFFECT OF VIOSTEROL ON THE CONCENTRATIONS OF SERUM CALCIUM IN INFANTILE TETANY

plication of, or is complicated by, an infection, the response to therapy is also often retarded, so that the calcemia does not reach relatively normal levels until intensive therapy has been instituted for a period or two or three weeks. For these reasons, it is difficult to appraise the efficacy of any therapeutic agent in tetany with any exactness; and

whatever comparison is made between antirachitic agents must be of a relative nature. When viosterol is thus compared to cod liver oil in infantile tetany, the former appears much superior in that the period of recovery is considerably shortened. Thus, using cod liver oil and calcium chloride, Hoag (41) found that in one of his patients the concentration of calcium in the serum rose only from 7.1 to 8.8 in a period of twenty-six days and to 8.7 mgm. per cent in thirty days; in another, the serum calcium concentration rose from 5.4 to 6.2 mgm. per cent in twenty days; and in a third, the concentration of serum calcium remained at 8.4 mgm. per cent after fourteen days of therapy. Similar results were obtained by Kramer and Howland (42), Bakwin (43), and Rosenstern (44). Rosenstern studied the effects of 25 cc. of cod liver oil on the clinical manifestations and electrical reactions of 22 infants with tetany and found that in 18 the reaction to cathodal-opening contractions returned after three weeks of therapy, and that, in some, the facial (Chvostek) signs persisted for more than a month after therapy was begun. Bakwin's results are of special interest since he and his associates used 12 cc. of *standardized* cod liver oil, as against the oils of uncertain potency used by Rosenstern in 1910, and possibly also by Kramer and Howland in 1920, and by Hoag in 1923. Bakwin found that this dosage of the oil was "inadequate in the treatment of tetany of infants, its action being too slow and too inconstant." Hess (19) expressed a similar opinion in the following sentence: "The action of cod liver oil [in tetany] is slow, as it is in rickets; indeed, its main action seems to be on the rickets rather than directly on the tetany."

With respect to viosterol, our results agree with those obtained by Hess (19), György (10), and Bakwin (43) with the older preparations of irradiated ergosterol. Bakwin found irradiated ergosterol to be far superior in its calcium-raising properties in tetany to either cod liver oil or radiations with the mercury quartz lamp; and Hess (19), concurring with this view, stated: "*In tetany, activated ergosterol is characterized by its rapid action.*"

In addition to the cases of active tetany in which calcium salts were administered along with the viosterol, there were also 26 cases with definite hypocalcemia without tetany treated with viosterol alone. In most of these, the hypocalcemias were associated with rickets, and

the effects of viosterol were made evident by the prompt increases in the serum calcium to normal levels and by the deposition of lime salts in the rachitic metaphyses.

12. EFFECT OF VIOSTEROL ON THE CONCENTRATIONS OF CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM

In the course of our study, several hundred determinations for calcium and inorganic phosphorus were made on blood serum. The samples of blood were obtained mostly from babies suffering from rickets, tetany, or from both, before and after the institution of therapy with viosterol, and also from patients receiving viosterol as a prophylactic measure against rickets. In the analyses for calcium, the methods of Kramer and Tisdall (45) and of Fiske and Logan (46) were used; and for the determination of inorganic phosphorus, the procedure of Fiske and Subbarow (47) was followed. In the method of Kramer and Tisdall, using $KMnO_4$ to titrate the precipitated calcium oxalate, the figures for calcium in serum are somewhat higher than those obtained by the Fiske and Logan procedure, and hence our so-called "normal" figures are scattered more widely than one would expect from using one or the other method exclusively. Thus, instead of considering 10.0 to 11.5 mgm. per cent of calcium as the normal range for infant sera, we are inclined to believe that a concentration of serum calcium of 12.0 mgm. per cent, or even higher, is within the realm of the normal, especially in children receiving adequate amounts of vitamin D.

The efficacy of viosterol in raising the levels of serum calcium from hypocalcemic to normal levels has already been discussed under "Tetany." The effects of various dosages of viosterol on the concentrations of calcium in the serum at various intervals of time after therapy was begun, are illustrated in figure 22. It is seen that in most instances the concentrations of serum calcium were below 12 mgm. per cent; that a few were between 12 and 13; and that but two were higher than 13 mgm. per cent. Of the last two, one occurred in a patient with late rickets receiving daily exposures from an ultraviolet lamp in addition to 20 drops of viosterol; and when the heliotherapy was suspended and the viosterol continued, the concentration of serum calcium fell, within two weeks, to 9.3 mgm. per cent. Since none of

our patients showed clinical or other laboratory evidence of ill health, the *relatively* high calcium values in the blood either denote improved retention or are due to the drawing of blood soon after a feeding, *i.e.*, postprandial hypercalcemia.

The concentrations of inorganic phosphorus in the blood fluctuated even more than did the levels of calcium. In rachitic children with hypophosphatemia, viosterol usually produced a prompt increase in the levels of inorganic phosphorus in the serum within a period of a

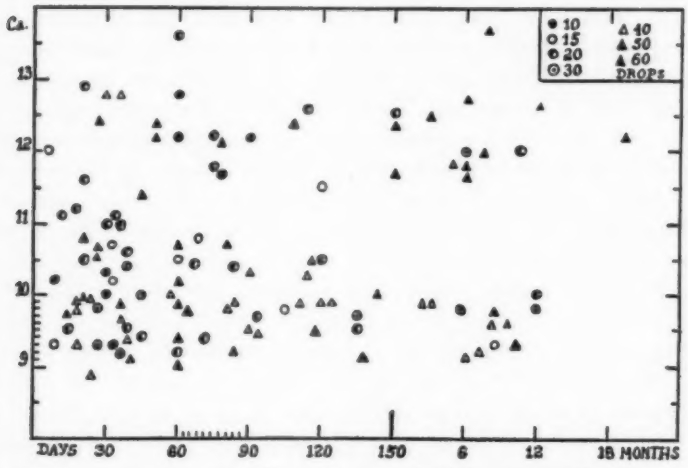

FIG. 22. CONCENTRATIONS OF SERUM CALCIUM (IN MILLIGRAMS PER CENT) IN PATIENTS RECEIVING VARYING DAILY DOSAGE OF VIOSTEROL FOR PERIODS INDICATED

few days; and in patients with hypocalcemia and relative hyperphosphatemia, it frequently caused a slight decrease in the concentrations of inorganic phosphorus while the calcium levels were increasing, but more often the hyperphosphatemia was sustained for some time. In only one instance did viosterol fail to increase materially the concentration of inorganic phosphorus in the serum. This failure occurred in a girl of ten years suffering from "late" rickets, in whom the rickets was little affected by irradiation from the mercury quartz lamp and 20 drops of viosterol daily through a period of forty-five days. During

this time, the concentrations of serum inorganic phosphorus rose only from 2.3 to 3.2 in 15 days and to 2.9 mgm. per cent in forty days. As explained elsewhere (29), had we given this patient larger amounts of viosterol, the rickets might have responded to therapy and the serum inorganic phosphorus increased to higher levels.

The use of viosterol for relatively long periods produced but few high levels of inorganic phosphorus in the serum. The higher concentrations were usually encountered in patients suffering from tetany associated with initial hyperphosphatemia and in patients receiving supplements of sodium or of calcium phosphate in addition to the viosterol. The highest level of inorganic phosphorus occurred in a patient with tetany showing initial concentrations of calcium and inorganic phosphorus of 5.9 and 8.0 mgm. per cent, respectively. After one month of therapy with calcium chloride and 15 drops of viosterol daily, the calcium increased to 10.2 and the inorganic phosphorus to 9.7 mgm. per cent. However, in spite of this relative hyperphosphatemia, he showed no symptoms of tetany or other ill effects. The concentrations of inorganic phosphorus in cases uncomplicated by tetany, or by supplemental therapy with phosphate, ranged between 4.3 and 7.8 mgm. per cent, even when the viosterol was taken in doses of 40 to 60 drops daily for some months.

13. INDICATIONS AND CONTRAINDICATIONS FOR VIOSTEROL IN DISEASES OTHER THAN RICKETS AND TETANY

In addition to the use of viosterol in the prevention and treatment of rickets and tetany, its use has been extended to other maladies in which there is, or might be, a disturbance in the metabolism of calcium and phosphorus. In some of these diseases, the metabolic derangement is quite definite, and hence vitamin D could be expected to exert a beneficial effect; in others, the relation to calcium and phosphorus metabolism is indirect; and in still others, the underlying cause is not metabolic but rather developmental, *i.e.*, an inborn fault in bone-producing tissue, and in such cases vitamin D could hardly be expected to be of any value. In the first category may be included: Celiac disease, sprue, general osteoporosis, fractures, osteomalacia, renal rickets, parathyroprivic tetany, pregnancy, and dental caries. In the second group may be enumerated: Lead poisoning, tuber-

culosis, and the allergic diseases. In the third group may be listed: Osteogenesis imperfecta and the dyschondroplasias, *i.e.*, achondroplasia and Morquio's disease (osteochondrodystrophy deformans). We have not employed viosterol in all the diseases mentioned; but in those in which we have used it, the number of cases in each group was rather small. Accordingly, the discussion is limited to the use of viosterol in celiac disease, renal rickets, parathyroprivic tetany, and lead poisoning. We are especially desirous to call attention to the fact that vitamin D may be contraindicated, under certain conditions, in the last three maladies. The reasons for these contraindications have been discussed in greater detail elsewhere (48, 49, 50).

(a) *Celiac disease.* The gross metabolic disturbance in celiac disease is a loss of fat by way of the bowel. The constant loss of fat by this route may result also in a loss of much calcium in the form of calcium soaps, and thus lead to rickets, osteoporosis, and sometimes to tetany. It is, therefore, just as important to prevent these metabolic changes as it is to treat the disease itself. Cod liver oil cannot always be used effectively as a source of vitamin D because of its large bulk of fat; and most clinical observers believe that fat should be restricted in the diet of patients suffering from this disease. It is also possible that the vitamin D from cod liver oil is poorly absorbed, owing to the rapid elimination of fat by the lower intestinal canal. On the other hand, viosterol, because of its high concentration of vitamin D per unit volume as compared with cod liver oil, seems to be an ideal antirachitic agent in this malady. We had occasion to use it, in conjunction with dietary therapy, in a two-and-one-half year old patient who showed rickets, osteoporosis, and a moderate hypocalcemia, and in three other patients as a prophylactic against the development of these complications. In the first patient, 40 drops of viosterol daily cured the rickets and the osteoporosis within four months, and the concentrations of calcium and inorganic phosphorus rose from initial levels of 8.1 and 2.5 mgm. per cent to 9.5 and 6.0 mgm. per cent, respectively, at the end of three months of therapy. The x-ray appearance of the bones of this patient before and after therapy was included in a previous publication on "Late Rickets" (27). In the other three patients, the moderate doses of viosterol prevented the occurrence of rickets and osteoporosis, which probably would have been present had viosterol not been given.

(b) *Renal rickets.* Although we have seen several cases of renal rickets in the past six years, we have refrained from using vitamin D in this form of rickets. Indeed, we have cautioned against its use on previous occasions (27, 48). It is now well established that patients with this condition usually have retention of phosphorus and are unable to excrete it in the urine. The excretion of phosphorus as the insoluble calcium salt by way of the feces may result in a high-phosphorus, low-calcium type of rickets. In such cases, the constant transport of calcium phosphate in excess of its solubility may lead to abnormal calcification in the soft tissues, especially in the medial coats of the medium-sized arteries. Since vitamin D increases not only the calcium but also the inorganic phosphorus concentration in the blood, the injudicious use of vitamin D preparations may enhance the abnormal calcification. Shelling (51) and Shelling and Asher (52) showed that animals retaining excessive amounts of phosphorus, either through the feeding of diets high in phosphorus or as a result of parathyroidectomy, are more susceptible to abnormal calcification and to the toxic effects of vitamin D than are normal animals; and Mackay and Oliver (53) reported the occurrence of calcification in the kidneys of animals fed diets rich in phosphorus without excessive amounts of vitamin D. Since patients with renal rickets are susceptible to metastatic calcification even though vitamin D is not administered, as in the cases reported by Smyth and Goldman (54) and by Shelling and Remsen (55), the use of viosterol or even of weaker antirachitics, such as cod liver oil, is definitely contraindicated. The occurrence of arterial calcification in Karelitz's (56) patient with renal rickets treated with Vigantol is, therefore, no indication that these lesions were due to vitamin D overdosage, since they occur quite often in patients not so treated. From the chemical findings in the blood and excreta, it would seem that the logical treatment of this type of rickets is to abstain from the use of vitamin D and to feed diets high in calcium and relatively low in phosphorus, so as to divert the retained phosphate into the colon as the insoluble salt of calcium.

(c) *Parathyroprivic tetany.* Since the advent of vitamin D as an antirachitic and antispasmodic in the rickets and tetany of infants, its use has been extended by some also to parathyroid tetany. Although vitamin D is capable of elevating the concentrations of calcium in the serum of normal and tetanic individuals, yet its use in hypo-

parathyroidism is not without danger, since it also increases the concentration of inorganic phosphorus and thus accentuates the phosphate retention. More recent studies on the physiology of the parathyroids indicate that there is a considerable difference in the behavior of vitamin D and parathyroid hormone, not only in the normal individual, but in the parathyroprivic organism. Thus, while one of the effects of vitamin D is to cause the body to *retain* phosphate, the function of the parathyroids, or of the hormone extracted from them, is to *reduce* phosphate retention (57). Since the retention of phosphate in the blood is an important factor in the pathogenesis of parathyroid tetany, the use of therapeutic agents which might accentuate its retention is not advisable. An example of this difference in the effects of the two substances in parathyroprivia is illustrated in a previous publication (48). It shows that the administration of viosterol to a patient with hypoparathyroidism produced an elevation of the levels of both calcium and inorganic phosphorus and a retention of phosphate, as indicated by the absence of phosphorus from the urine; whereas parathyroid hormone increased the concentration of calcium and decreased that of phosphorus by producing a "phosphate" diuresis. The adverse effects of phosphate retention were also evident from the fact that tetany developed soon after viosterol was withdrawn, owing to the fact that the concentration of calcium in the serum was decreased while that of the inorganic phosphorus was sustained. For these reasons, we have used viosterol in parathyroid tetany with the greatest of caution—only in relatively small amounts and in conjunction with high-calcium, low-phosphorus diets. In the past three years we have been able to maintain two patients with parathyroid tetany on merely a low-phosphorus, high-calcium diet, without the use of either parathyroid hormone or vitamin D. The differences in the effects of vitamin D and parathyroid hormone have been discussed more fully elsewhere (49).

(d) *Lead poisoning.* The metabolism of lead simulates that of calcium in that it is capable of forming insoluble compounds with phosphate. This similarity has, however, been misinterpreted in its application to the therapy of lead poisoning. Thus, while defective ossification may be brought about by disturbing the ratio of calcium to phosphorus of the diet in either direction, *i.e.*, by rendering the

calcium level high and the phosphorus low, or *vice versa*, students of lead poisoning have applied only part of this knowledge to the therapy of this malady, namely, the result and consequences of low calcium feeding. The fact that additions of calcium to diets low in calcium usually prevents and cures the osteoporosis, which results from such deficient rations, led these investigators to conclude that supplements of calcium to diets in general are also conducive to improved ossification; and since lead behaves chemically like calcium, the latter was advocated in the treatment of lead poisoning as a means of "driving" the lead into the bones where it was supposed to be stored, along with the calcium, in an innocuous state. However, it is now a well established fact that the addition of calcium salts, other than the phosphate, in amounts sufficient to increase the ratio of calcium to phosphorus beyond normal limits, would result, not in improved calcification, but, on the contrary, in deficient deposition of lime-salts in the bones. The poor state of ossification is probably due to an attempt on the part of the body to excrete the excess calcium ingested as the insoluble calcium phosphate into the bowel, and in this way the body is robbed of phosphate necessary for calcification. The production of rickets in rats is accomplished precisely by this means, namely, by diets in which the phosphorus is low and the calcium content is about four times that of phosphorus. In order to prevent the development of rickets in rats, without the addition of vitamin D, the ratio of calcium and phosphorus in the diet is usually changed, either by removing part of the calcium or, better, by adding phosphate, so that the two elements approach a more nearly physiological relationship. Obviously, the injudicious use of calcium does not always lead to superior calcification but rather to the opposite effect, especially when the phosphorus intake is inadequate; and if a definite ratio between calcium and phosphorus is requisite for calcification, this ratio must be maintained also if lead is to be deposited in the bones, *i.e.*, there must be sufficient amounts of phosphate to enable the lead to be deposited as the insoluble *lead phosphate*. That this mechanism operates *in vivo* was proved experimentally in rats (50), and the results so obtained were subsequently applied to the therapy of lead poisoning in human beings. The experiments with rats clearly indicated that the additions of calcium carbonate to an optimal stock diet containing lead car-

bonate resulted in an increase in toxicity, whereas sodium phosphate *prevented* the effects of the ingested lead. The results with phosphate therapy in human beings have likewise been more satisfactory than with other forms of therapy, except in advanced cases of lead encephalopathy in which cases most forms of treatment are without avail.

As vitamin D is capable of preventing rickets in rats, regardless of the calcium intake, it was expected that under similar dietary conditions, it would also promote deposition of lead in the bones. Experimentally, however, this was not the case. It was found (50) that animals receiving viosterol in a high-calcium, lead-containing diet, lost weight at a more rapid rate and died much sooner than their mates who received no viosterol; whereas the animals subsisting on the lead rations containing sodium phosphate showed no deleterious effects of the vitamin D therapy. This peculiar behavior of vitamin D was rather surprising, but we have subsequently found that vitamin D increases not only the concentration of calcium and inorganic phosphorus in the blood but also that of lead, thus exposing the organism to a greater concentration of *circulating* lead. Accordingly, we have abandoned the use of vitamin D in lead poisoning, especially when we have reason to believe that the dietary regimen with regard to phosphate will not be carried out at home. It is interesting in this connection that the number of cases of acute lead poisoning admitted to our clinic is greatest during the summer months, when the sun's rays emit an abundance of ultraviolet, than in any other season of the year. A similar seasonal incidence of lead toxicity in rats was found by Krehbiel and coworkers (58) with colloidal lead-manganese phosphate. In the winter, their rats tolerated 4 mgm. of this preparation per 100 grams of body weight; whereas in the summer 3 mgm. proved to be toxic.

These findings suggest that in the treatment of lead poisoning vitamin D should be used cautiously, and only when the diet is not excessive in calcium as compared with phosphorus. We have used viosterol in clinical cases of plumbism only in conjunction with disodium acid phosphate (3.0 gm. daily) and only when it was advisable to divert the lead from the blood into the osseous tissue, but not when it was desired to delead the patients. For the latter pur-

pose, we have employed a diet high in phosphorus and low in calcium without additions of vitamin D. In this way the lead abstracted from the bones is excreted as lead phosphate.[10]

14. THE ALLEGED TOXICITY OF IRRADIATED ERGOSTEROL

In 1928, shortly after the introduction of irradiated ergosterol as an antirachitic agent, Pfannenstiel (59) and Kreitmair and Moll (60) reported that "enormous doses" produced pathologic lesions in the soft tissues of a variety of species of animals—some being more susceptible than others. Their results were corroborated by numerous investigators, many of whom, like Pfannenstiel, and Kreitmair and Moll, failed to state the antirachitic potency of the irradiated ergosterol used, except that it was given in so many milligram doses. In the experiments in which the dosages are stated, the range between the toxic and the therapeutic dose appears to be quite wide. Collazo, Rubino and Varela (61) found that rats died within six weeks when fed the McCollum rachitogenic diet no. 3143 and 50,000 times the therapeutic dose of irradiated ergosterol. Harris and Moore (62) and Light, Miller and Frey (63) observed that 100,000 times overdosage was fatal to rats, whereas 10,000 times was not toxic even when fed for as long as six months. Similar results were obtained by Bills (64), Harris (65), Hoyle (66), Shelling (51, 52), Jones (67), Duguid (68), and others, who, in addition, stressed the importance of the calcium and phosphorus of the diet as a factor in the toxicity of irradiated ergosterol.

In human beings, the margin of tolerance has not been definitely determined. In the earlier reports on the toxic effects produced in infants by irradiated ergosterol, the dosages are given in terms of milligrams, instead of "rat" units, so that the exact antirachitic potency of these substances cannot be determined. With regard to *standardized* irradiated ergosterol, as produced in America, there are

[10] Dr. Irving Gray of Brooklyn, New York, informs us that he has used this form of therapy in deleading more than fifty patients suffering from chronic, industrial lead poisoning, and that in none of these has he seen symptoms of toxicity during the treatment, in spite of the fact that the excretion of lead increased in most instances. A preliminary report of his investigation appeared in *J. A. M. A.*, **104**, 200, 1935.

several reports from which it may be inferred that in human beings the toxic dose is also quite remote from the therapeutic level. Thus Hess, Poncher, Dale and Klein (9) gave as much as 40 times the therapeutic dose of viosterol to babies for six months and noted no untoward results other than a tendency to constipation and colic. Reed and Seed (69), and Rappaport and Reed (70) gave 300 times the ordinary dose of viosterol to normal individuals and to patients afflicted with parathyroprivic tetany. In the latter, the tetany was alleviated and no toxic manifestations developed; but in some of the individuals with intact parathyroids, mild symptoms developed when the administration of viosterol was continued for several weeks. Crimm and his associates (71) fed 150 times the usual dose of viosterol to normal and to tuberculous individuals subsisting on diets fairly rich in calcium and found no toxic manifestations, except very mild symptoms in a few patients subjected to the therapy for a relatively long time.

The lethal dose for human beings is also unknown. The known largest doses are those used by Reed (69) and by Rappaport and Reed (70) in patients suffering from asthma and other ailments, and by Spies and Hanzal (72) in patients about to die of incurable maladies. The dose used by the former was calculated by Bills (73) as 920 times the ordinary therapeutic dose of irradiated ergosterol; and the amount used by the latter amounted to 6,000 times the usual dose. Hypercalcemia developed in both instances, but symptoms of serious poisoning were not noted in Reed's patients, and pathologic calcification was not found when Spies and Hanzal's patients were examined *post mortem*.

It is of interest that the reports on the range between the therapeutic and toxic dose of the American-made, *standardized*, irradiated ergosterol differ materially from those of the German product "Vigantol." The reports on the toxic effects of vitamin D came mostly from Germany, but Hess and Lewis, in New York, also reported adverse effects from the use of vigantol. The potency of this substance, which was presumed to be vitamin D, was expressed in *milligrams*, and dosages of about 1 to 5 milligrams were recommended for the healing of rickets; yet, when as little as 5 milligrams of Vigantol was given, some children reacted with a peculiar train of symptoms—anorexia, vomiting, diarrhea, and hematuria: and in some instances the

therapy is said to have caused death. According to Bills' calculations, 5 milligrams of Vigantol could hardly supply enough *vitamin D* to account for the observed effects, since pure calciferol contains only 40,000 International antirachitic units per milligram, and the potency of 1 milligram of unpurified irradiated ergosterol in the form of Vigantol was probably only a small fraction of that amount.

What then caused the toxic effect of Vigantol, and why are accounts of the toxicity of vitamin D in children no longer reported, in spite of the fact that irradiated ergosterol has been used, in some instances, in fairly large doses and for relatively long periods of time? In 1928, Dixon and Hoyle (74), and Underhill (75) suggested that the toxic action of Vigantol was due to the presence in it of a toxic by-product, the result of the irradiation of the ergosterol in alcohol, as in the German method, instead of in oil, as in the English method. This possibility, however, was denied by Harris (76). In 1930, Holtz (77) and Windaus (78) also supposed that irradiation produced two distinct substances—vitamin D and a "calcifying" toxic material. In 1931, Göttche and Kellner (79) established the important point that the difference between the toxic and the therapeutic dose of Vigantol was quite narrow. Like Dixon and Hoyle, they too suggested that the high toxicity of Vigantol was due to some by-product of irradiation, but they were unable to prove it. Bills (73), however, has recently suggested that the toxic substance in Vigantol may have been *toxisterol or substance 248*, and not vitamin D or calciferol, the former substance being a by-product of irradiation of ergosterol in alcohol, as first suggested by Dixon and Hoyle. The subject has been discussed fully in a recent review by Bills (2). He states that when ergosterol is irradiated in alcohol, instead of in ether or oil, as in the English or American methods, and the irradiation is considerably prolonged, toxisterol is formed in amounts out of proportion to the vitamin D it contains. Toxisterol shows an intense ultraviolet absorption band at 248 mμ instead of at 265 mμ, as does pure calciferol, and hence the designation "substance 248." Over-irradiation of ergosterol in alcohol was precisely the method patented by the manufacturers of Vigantol, which, therefore, presumably was a mixture of toxisterol and vitamin D. This method, however, has now been abandoned, so that vigantol no longer contains this toxic substance.

Notwithstanding the fact that Vigantol probably produced some

toxic effects in human beings, it is equally true that many of the reports are groundless and the clinical and pathologic findings purporting to show its toxic effects are so slight and insignificant as to be within the realm of the normal or to have no etiologic relation to vitamin D. As with all new therapeutic discoveries, so with vitamin D. Its praises and condemnations run in cycles. For example, before the introduction of vitamin D, slight cellular infiltration of the wall of the kidney pelvis of a child suffering from chronic pyelitis would be looked upon as a natural and expected finding; yet, as reports of toxicity of Vigantol were forthcoming, such, or similar findings, assumed sufficient significance in the eyes of investigators as to be thought worthy of record as signs of poisoning by vitamin D. Authentic incidences of poisoning with therapeutic doses of *standardized* viosterol have not been reported, but there still exists some fear in the minds of clinicians that even therapeutic amounts of viosterol might be injurious to human tissue. In our six years' experience with standardized viosterol in several hundred children, we have not seen a single case in which clinical manifestations of overdosage were evident, in spite of the fact that in many of these children the dose was 10 to 12 times that usually employed for the prevention of rickets, and the period of administration, in some, was more than two years. In addition, we were able to examine the tissues of 27 children who had received moderate dosages of viosterol for varying periods. They died from a variety of causes, such as dysentery, pneumonia, tuberculosis, and so forth. The necropsy report of 13 of these cases appeared elsewhere (80); and since the publication of this report, 14 additional cases have been studied. In none of these cases was there evidence of tissue damage which might have been attributed to viosterol therapy, and only in one patient afflicted with disseminated tuberculosis since infancy, who received 30 to 40 drops of viosterol from the sixth month of life until his death at eighteen months of age, did calcification occur in tissues other than the bones. In this instance, the calcification occurred in the tuberculous lesions in the lungs—a sign of healing which we anticipated would be caused by this moderately large dosage of viosterol, together with a diet high in phosphorus.

The osseous tissues of the entire group of children were examined radiologically and histologically, and, like the soft tissues, they too

failed to show any abnormalities. The bones of the children who had had rickets and who had received viosterol as a curative measure showed complete healing of the rachitic process; and the bones of those who received this substance as a prophylactic against rickets showed normal calcification. The curative group contained three children; and the prophylactic group contained twenty-four, seventeen

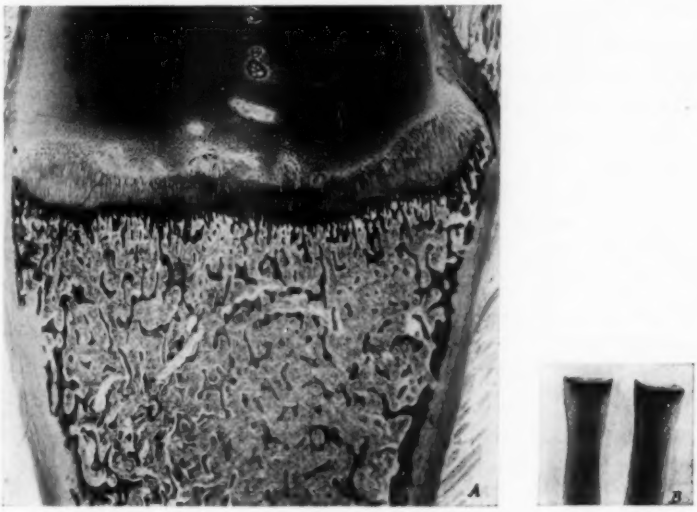

FIG. 23. (*A*) HISTOLOGIC AND (*B*) X-RAY APPEARANCE OF RIB IN AN EIGHTEEN-MONTH-OLD COLORED CHILD WHO HAD PREVIOUSLY HAD RICKETS AND HAD RECEIVED 15 TO 20 DROPS OF VIOSTEROL DAILY FOR TEN MONTHS

Note the heavy calcification in the cartilage and the thickness of the lattice bordering on the cartilage. Histologically, a normal, well calcified bone.

of whom were prematurely born and received from 10 to 25 drops of viosterol daily for periods of from six weeks to one year. Rickets was absent in the entire group, as well as in a colored child who received only 5 drops of viosterol daily from the first month of life until his death at fourteen and one-half months. The histological and roentgenological appearances of a rib of a colored child (H. N.) who had previously had rickets and had received 15 to 20 drops of viosterol

for ten months are shown in figure 23; those of a full-term colored child receiving only 5 drops of viosterol as a prophylactic measure are shown in figure 3; those of a colored premature infant receiving but 20 drops of viosterol for eleven months are illustrated in figure 5–D; and those of a ten-month-old white girl receiving 20 drops of viosterol daily for five months are shown in figure 4. These illustrations show that these amounts of viosterol were sufficient to cure or prevent rickets in these colored babies, including the prematurely born, and that doses of viosterol of several times the recommended amounts were without detriment to the osseous tissue even when administered for many months. Indeed, some of these bones showed what Göttche and Kellner (81) consider "superior" calcification, *i.e.*, heavy deposition of lime salts in the cartilage and relatively thick lattice bordering on the cartilage. Göttche and Kellner also called attention to the fact that the dense ends of bones observed in x-ray films have been erroneously mistaken by some investigators for overdosage with irradiated ergosterol, whereas, in reality, they were due to feeding "elementary" phosphorus along with the vitamin. As a typical example of this misconception, they cite the case described by Opitz (82) in which Vigantol, elementary phosphorus in cod liver oil, and exposures to the ultraviolet lamp were all given daily.[11] Since the feeding of elementary phosphorus alone produces such sclerosis at the ends of the bones (83), the dense ends of the bones observed in x-ray films were not necessarily due to overdosage with vitamin D.

15. VIOSTEROL AS COMPARED WITH COD LIVER OIL IN THE PREVENTION AND TREATMENT OF RICKETS

As already mentioned, the notion that the therapeutic ratio between cod liver oil and commercial viosterol for human beings is 4:1 has not been proved. Thus, if 3 teaspoonfuls of cod liver oil, containing about 400 Steenbock or 1,200 International units, is the average dose for the prevention of rickets in colored infants, then the efficacy of 5 drops of viosterol, containing only about 375 Steenbock or 1,125 International units, compares well with that of cod liver oil. In fact, students of

[11] A reproduction of the X-rays of the bones of this case is shown as an example of hypervitaminosis D in the latest edition of Pfaundler and Schlossmann's System of Pediatrics.

rickets have frequently commented upon the inadequacy of even three teaspoonfuls of cod liver oil to completely protect against rickets in some children; and we have observed failure in protection on many occasions. Indeed, in many of the children included in our groups of rickets and of tetany, the parents claimed that cod liver oil had been given regularly during the period preceding viosterol therapy, although it is quite possible that the amounts prescribed and actually taken did not coincide. The laxity on the part of mothers in administering cod liver oil regularly is due largely to its disagreeable odor and taste. Our social workers find that such laxity seldom exists in the case of viosterol, and hence viosterol is given much more regularly. Therefore, the superiority of viosterol over cod liver oil lies in the ease with which the former is taken by most babies.

In the case of *very severe* rickets, the failure of ordinary doses of cod liver oil to effect a complete cure is a well established fact. This failure is due to inadequate dosage; but, unfortunately, this inadequacy cannot be overcome by giving large amounts of cod liver oil, and one has to resort to remedies which contain vitamin D in greater concentrations. One of the most potent antirachitics is *irradiated ergosterol*. Our experience with viosterol as a curative agent in severe rickets coincides with that of the late Dr. Alfred T. Hess (19) who stated: "In almost every instance, the new therapeutic agent (irradiated ergosterol) has been found to be reliable in the cure as well as in the prevention of rickets. It has been especially remarkable in its rapidity of action—the signs of rickets disappearing more quickly than has been accomplished hitherto by means of cod liver oil, its concentrates or even by direct irradiation."

Viosterol differs from cod liver oil in that it does not contain vitamin A. At one time, the antixerophthalmic and the antirachitic vitamins were thought to be the same—both being fat-soluble, but the important observations of McCollum and his coworkers established the important fact that the vitamin preventing keratomalacia of the eye and the one protecting against rickets are separate and distinct. Even before this difference was recognized, it was known that the exclusion of fat-soluble vitamins from the dietary of experimental animals led to ophthalmia and to keratinixation of epithelial tissue and that these changes could be prevented by feeding butter fat or cod

liver oil. These tissue changes in the eye and epithelial linings predispose to an invasion of microorganisms; and since the primary keratinization and the secondary infection may be *prevented* by the administration of vitamin A, the notion became prevalent that this accessory food substance, vitamin A, was the "anti-infection vitamin" (84, 85). These experiments on lower animals have been carried over *in toto* by some, to the problem of the prevention and cure of infections in human beings, with the result that substances containing this vitamin are being advocated, if not exploited, extensively, without adequate experimental proof, for the prophylaxis of all sorts of infections, especially those of the respiratory tract. Although the need of vitamin A in the dietary of human beings, and especially of growing infants, is fully recognized by clinicians, yet, there is no adequate experimental evidence to show that additions of extra vitamin A to the diets of infants, older children, and adults will prevent or cure infections, since many of their articles of food contain liberal amounts of this substance.

There is no question that gross errors of diet, extreme malnutrition such as occurs in marasmic infants, or the inability to absorb or store vitamin A may lead to symptoms of vitamin A deprivation; but these are extreme conditions which occur but rarely, especially in modern times. The deprivation of butter fat from the dietary of infants and children, as it was practiced in Denmark during the World War, is seldom duplicated in America, unless the removal of butter fat from the milk is done for a therapeutic reason, in which case other vitamin A preparations are usually introduced into the dietary. In the diet of the average infant or child there is, therefore, no reason to believe that there is a deficiency of vitamin A or that the administration of additional vitamin A will prevent or cure infections. The experimental evidence of such claims has recently been analyzed by Clausen (86) who points out the numerous fallacies inherent in such investigations and conclusions and the necessity of more adequately controlled studies. The more recent studies of Wright (87) and of Hess and his coworkers (88) in infants, and of Shibley and Spies (89) in young adults attest to Clausen's conclusions. Their experiments show conclusively that there is no basis for the alleged protective value of vitamin A in infections, since such therapy had no effect on their incidence or severity.

The fact that the incidence of infections is determined by so many other factors, such as, climate, season, exposure, contagion, and the state of the physical and mental health, militates against the possibility that infections could be prevented by a single vitamin.

We did not study systematically the incidence of infections in groups of infants receiving either viosterol or cod liver oil, but the fact that a large percentage of the children admitted to the Harriet Lane Home dispensary and wards with severe infections received adequate amounts of cod liver oil, and also the fact that many of the children in our viosterol group who were recently examined for the condition of their dentition were found to have been in perfect health since infancy and comparatively free from infection makes us believe that the value of *additional* vitamin A as an agent against infections has been overestimated.

16. SUMMARY

The efficacy of viosterol as a preventive and curative of rickets and tetany was studied in several hundred cases over a period of six years. The diagnosis or exclusion of rickets was based on the physical examination, the roentgenological study of the bones, and, in many instances, on the determination of the blood serum for calcium and inorganic phosphorus. In some cases, these findings were corroborated by histological examination of the bones. The findings may be summarized as follows:

1. In full-term infants, including those of the colored race, a daily dose of 5 drops of viosterol, containing about 1,125 International units, was found to be efficacious in preventing rickets.

2. In prematurely-born infants and in babies growing at too rapid a rate, mild degrees of rickets appeared only in a few babies who received 5 or 10 drops of viosterol daily and no evidence of the disease in those receiving larger doses. Accordingly, it may be advisable to administer 15 to 20 drops of viosterol daily for the first three to four months of life, after which time the dose may be reduced to that of full-term infants.

3. In 134 cases of active rickets in which it was possible to estimate the healing time by means of x-ray films, the efficacy of a given dose of viosterol was found to be related largely to the severity of the

disease. Mild cases healed within one and one-half to two months even on small or moderate doses of viosterol, whereas severe cases healed completely only after three to five months of therapy with comparatively larger doses of viosterol. The average duration required for complete healing in the entire group, comprising all degrees of rickets and dosages of viosterol, ranging from 15 to 60 drops daily, was 3.7 months. In the group receiving 10 drops of viosterol daily, the healing time was 5 months; in the group receiving 15 drops it was 4.3 months; and in the group receiving 20 drops it was 3.1 months. In the groups receiving 30 to 60 drops of viosterol daily the average healing time was not shortened, indicating that larger amounts than 20 drops of viosterol daily are not always more efficacious in inducing more rapid healing. The larger dosages, however, were usually given to children with late rickets or with very severe infantile rickets in whom smaller amounts of vitamin D would have accomplished much slower healing. In the infants with very severe rickets, the more rapid healing brought about by the larger amounts of vitamin D prevented the development of deformities of the chest and extremities.

4. Total refractoriness to viosterol was not encountered in the entire group, and partial refractoriness was seen in but one case, that of a girl of ten years, treated with 20 drops of viosterol for a period of only forty-five days.

5. In infantile tetany, viosterol, in conjunction with calcium salts, alleviated the symptoms of tetany in more than 100 cases, and increased the concentration of the blood to normal levels in those in which chemical analyses of the blood were made. In some patients with hypocalcemia without evident tetany, viosterol produced an increase in the concentration of serum calcium without the addition of calcium salts.

6. In our entire six years' experience with its use, toxic manifestations of viosterol were never encountered clinically, in spite of the fact that in some children it was given in doses of 30 to 60 drops daily for many months. Evidence of toxicity was likewise absent in the tissues of 27 children examined *post mortem*.

7. The uses and misuses of viosterol in diseases other than rickets and tetany are enumerated and discussed.

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EXPERIMENTAL AND PATHOLOGICAL STUDIES ON THE PATHOGENESIS OF ACUTE HAEMORRHAGIC PANCREATITIS

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INTRODUCTION

In current text-books of pathology, there is a great deal of speculation and a striking lack of agreement regarding the etiology and pathogenesis of acute haemorrhagic pancreatitis (1, 2, 3, 4, 5, 6, 7, 11). Excluding haemorrhage into the pancreas resulting from primary arterial disease (the so-called "pancreatic apoplexy"), all authors agree in assigning the origin of haemorrhagic pancreatitis to a cause extraneous to the pancreas itself, but beyond this point opinions differ. One theory, which receives but little support, supposes that intestinal juice may, under certain circumstances, be forced into the pancreatic duct from the duodenum, thus introducing infection and activating the ferments of the pancreatic secretion. The possibility is suggested that the pancreas may be infected by bacteria reaching it by way of lymph channels from a diseased gall-bladder, or possibly from more distant foci of infection by way of the blood stream. How-

ever, the theories which are favored by the majority of writers depend upon the existence of an anatomical arrangement of the duodenal orifices of the common bile duct and pancreatic duct of such a nature that the impaction of a gall-stone in the ampulla of Vater (8, 9), or spasm of the sphincter of Oddi (10), will direct the flow of bile from the bile duct into the main pancreatic duct. No good explanation is offered for the occurrence of haemorrhagic pancreatitis in persons in whom the bile duct and pancreatic duct open separately into the duodenum.

The anatomical conditions which would permit the conversion of the bile duct and pancreatic duct into a continuous channel after the impaction of a gall-stone in the ampulla of Vater are present in about 65 per cent of persons, according to Cameron and Noble (12), though others who have investigated this question have reported much lower figures (15). Whatever may be the frequency of this arrangement of the ducts, however, cases of haemorrhagic pancreatitis in which a gall-stone is found in the ampulla are admittedly uncommon (18). In only 1.4 per cent of the 437 cases in Guleke's series (13) was a gall-stone found in the papilla, while Schmieden and Sebening (14) reported the occurrence in only 4.5 per cent of their series of 1278 cases. Moreover, it must be borne in mind that, unless a very careful dissection is made, the mere presence of a calculus in the ampulla of Vater does not prove that the bile had access to the pancreatic duct. In the first place, the pancreatic duct may not have opened with the bile duct in the ampulla; and in the second place, even in the instances in which the ducts open together into the ampulla, unless the stone is minute and situated at the tip of the papilla, it will obstruct the pancreatic duct as well as the bile duct, as has been repeatedly shown (15).

Furthermore, it should be remembered that the presence of bile in the pancreatic duct at autopsy does not necessarily indicate that the bile entered the duct during life, for it is an almost universal routine practice to test the patency of the bile passages at autopsy by exerting pressure upon the gall-bladder while observing the papilla of Vater. This practice has been recommended by autopsy manuals from the early "Sektions-Technik" of Virchow (38) to those of the present time. Often, very considerable pressure has to be made upon the gall-bladder in order to force the bile from the papilla, even in cases in which the bile passages are normal; and in a case in which the bile duct and pancreatic duct open together into the ampulla, if there is a stone in the ampulla, bile may obviously be forced into the pancreatic duct by the pressure exerted upon the gall-bladder at autopsy, even though the intravitam pressure conditions may not have permitted its entry. It is therefore important that no pressure be exerted upon the gall-bladder in any case in which there may be a

question of an ante-mortem entry of bile into the pancreatic duct. It may be remarked that, even in the first reported case in which bile was found in the pancreatic duct in association with a stone in the ampulla of Vater (8), it is stated in the protocol that "by very firm pressure on the gall-bladder several drops of bile can be squeezed with difficulty into the duodenum"; and in the next reported case in which bile was found in the pancreatic duct (45) it is stated that increasing pressure was made on the gall-bladder until a calculus lodged at the ampulla was finally dislodged by the pressure and spurted out into the abdominal cavity. By clamping the tip of the papilla with small forceps and exerting pressure upon the gall-bladder we have had no difficulty in forcing bile throughout the pancreatic duct in routine autopsies in which the bile duct and pancreatic duct opened together into the ampulla.

In the absence of a stone in the ampulla of Vater, it has been suggested that, in the instances in which both the bile and the pancreatic duct open together in the ampulla, a flow of bile from the common duct might be diverted into the pancreatic duct during life by spasm of the sphincter of Oddi. Although Archibald (10) has carried out interesting experiments on the resistance of the sphincter in the cat to pressure within the biliary system, there is at present scant evidence (*e.g.*, the finding of bile in the pancreatic duct in the absence of a stone in the ampulla) that sphincter spasm ever causes a flow of bile into the pancreatic duct in either the cat or the human being under natural conditions; and the idea has been criticized by Mann and Giordano (15) on the ground that the sphincter in man is usually so arranged that its contraction will close both the bile duct and pancreatic duct completely, rather than convert them into a continuous channel. Nevertheless, this explanation is frequently invoked when no obvious cause of pancreatitis presents itself.

While there is disagreement as to the frequency with which bile can be held responsible for the development of acute haemorrhagic pancreatitis, all authors agree that if bile is injected into the pancreatic duct experimentally it can produce the condition, as was shown by Opie (8) in 1901, and since then by others, and as we have had occasion to verify in the present study. The experiments of Flexner (16, 17) and those of a number of other investigators demonstrate that the same result follows the injection of a wide variety of other irritants into the pancreatic duct. The various substances which have been used in such experiments are listed in the recent publication of Dragstedt, Haymond and Ellis (18). Since, in these experiments, quantities of from 4 to 30 cc. of bile or other fluids were injected forcibly into the pancreatic duct with a syringe, there can be no doubt, of course, that the duct-system of the pancreas or the pancreatic acini or both were ruptured by the injection. In our own experiments, we have shown,

by making injections of India ink into the pancreatic duct, that quantities of fluid smaller than any used previously in the production of haemorrhagic pancreatitis will rupture the small ducts or acini in even a large dog, leading to an immediate escape of ink into the interstitial tissues throughout a large part of the pancreas. Indeed, we know of no experiments in which haemorrhagic pancreatitis has been produced by the introduction of bile or any other substance in the absence of mechanical rupture of the ducts or acini. Archibald (42) was unable to produce necroses or haemorrhagic pancreatitis by the injection of small amounts (0.75 cc.) of bile into the pancreatic duct. Furthermore, Mann and Giordano (15) and Nordmann (19) have reported experiments in which the bile duct and the pancreatic duct were converted into a continuous channel and the free exit of the secretions into the duodenum prevented. In such experiments, although bile entered the pancreatic duct, the pressure was, apparently, insufficient to cause the rupture of the ducts or acini and, it is important to note, haemorrhagic pancreatitis did not occur.

Current theories postulate that bile entering the pancreatic duct in the human being produces necrosis of the pancreas because of its own irritating and necrotizing properties, or because it carries bacteria, or because it activates the inactive trypsinogen of the normal pancreatic secretion while the latter is still in the pancreatic ducts. Those who emphasize the rôle of infection introduced by the reflux of bile contend that necrosis of the pancreas is produced either through the direct action of the bacteria, or through the destructive effects of trypsin which follow upon the activation of trypsinogen by the bacteria or their products. All authors agree that the peculiar characteristics of the fat necroses which accompany haemorrhagic pancreatitis depend upon the action of pancreatic lipase, which is secreted in active form and which escapes into the tissues when necrosis of the pancreas occurs. Concerning the cause of the haemorrhage, opinions are not ventured so freely. Some believe that the haemorrhage is a result of a vasomotor reflex; others that it results from venous thrombosis; and others that it results from the erosion of the blood vessels by the pancreatic trypsin. Still others (18) regard it as improbable that trypsin can damage living cells. The various theories regarding the origin of the haemorrhage are discussed by Gruber (4).

A number of investigators have observed haemorrhages at the site of injection of trypsin solutions, but only a few have recorded any observations bearing upon the origin of these haemorrhages. Achalme (20) described local dilatation of capillaries with diapedesis of red blood cells following subcutaneous injections of a proteolytically active solution of "pancreatin." He found no changes in vessels larger than capillaries. Rosenbach (21) and Mück (22) followed with the microscope the sequence of events in the living frog's tongue after the injection of active commercial trypsin. They observed dilatation of capillaries, diapedesis of red cells, and finally, haemorrhages from ruptured capillaries. So far as we are aware, it has never been demonstrated that trypsin, acting alone, has the power to destroy the walls of previously intact arteries or veins, although there can be no doubt that the massive haemorrhage and the tearing of the tissues in haemorrhagic pancreatitis result from the rupture of vessels larger than capillaries.

From what has been said, it is obvious that there are numerous obscure points in the etiology and pathogenesis of acute haemorrhagic pancreatitis, and that the various theories that have been offered fail to explain satisfactorily the majority of cases that come under observation. Even the "bile" or "common channel" theory, which is more widely accepted than any other, is open to numerous objections. Some of these objections have been indicated above, and others are discussed in more detail in the papers of Mann and Giordano (15) and of others. It need not be denied that some cases of pancreatitis are caused by a retrojection of bile into the pancreatic duct. However, this seems to be the exception rather than the rule, and there is no doubt that many instances of acute pancreatic necrosis cannot possibly be explained on this basis. Dragstedt (18), after making all possible allowances in favor of the "common channel" theory, concludes that not more than 6 per cent of the cases of haemorrhagic pancreatitis are associated with a stone in the ampulla. He regards the evidence as indicating that about 54 per cent of the cases have occurred in individuals with "antecedent chronic disease of the biliary tract" but in the absence of a stone in the ampulla, and that in these cases either "we must assume that a common channel developed as a result of spasm or swelling of the sphincter of Oddi, or else that bile played no direct rôle." Even were one willing to accept the unproved assumption that in so large a proportion of the cases bile entered the pancreatic duct as a result of a spasm or swelling of the sphincter (an assumption which we certainly cannot regard as acceptable on the basis of the available evidence), there would still remain 40 per cent of the cases of haemorrhagic pancreatitis developing without any participation of bile. Into this latter category would obviously fall the many cases in which the bile duct and pancreatic duct open separately into the duodenum; so, too, the frequent examples of haemorrhagic pancreatitis localized in the body or in the tail of the pancreas, or limited as is sometimes the case, to the area drained by the duct of Santorini.

This large group of cases, the etiology of which the "common channel" theory cannot explain, and in which extension of infection from the bile duct to the pancreatic duct cannot be invoked, is usually dismissed by mentioning a variety of miscellaneous and relatively

rare causes such as trauma, hematogenous infection, gross obstruction to the outflow of pancreatic secretion with stasis and infection, the lodgment of an Ascaris in the mouth of one of the pancreatic ducts, thrombosis, embolism and so forth. However, the great majority cannot be so explained, and for these no satisfactory explanation has yet been offered.

The studies which we wish to present here bear particularly upon the etiology of the many cases of acute haemorrhagic pancreatitis which occur without evidence to incriminate bile, and for which no obvious cause is found at operation or at autopsy. The observations that we shall present upon the pathogenesis of the disease, however, and especially upon the cause of the haemorrhage in acute pancreatitis, find application in all cases regardless of etiology. An abstract of the results of a part of these investigations has been presented elsewhere (23).

EXPERIMENTS ON THE RUPTURE OF THE PANCREATIC ACINI BY RELATIVELY BLAND FLUIDS

Since haemorrhagic pancreatitis can be readily produced by injecting bile or other irritants into the pancreatic duct, preliminary experiments were carried out to satisfy ourselves of the correctness of our surmise that the injection of a like amount of any fluid into the duct will rupture the duct-acinar system mechanically.

These injections, and all other injections in these experiments, were made into the accessory pancreatic duct of the dog, which opens into the duodenum at a distance from the bile papilla and which, in the dog, is larger than the main pancreatic duct. The latter opens into the duodenum usually in company with the bile duct, but is quite small (24). With the animal under ether anaesthesia, the duodenum was opened by a longitudinal incision exposing the mouths of the ducts, and the injection was made from a syringe by inserting the tip of the blunt syringe-needle into the mouth of the accessory duct. Great care was taken not to handle the pancreas itself during the whole procedure. Since, in the dog, the two pancreatic ducts communicate in the interior of the pancreas, some of the fluid injected into the accessory duct frequently escaped from the mouth of the other duct.

The first experiments were performed on 6 dogs which were anaesthetized during the injections and killed shortly afterward. India ink was injected into the accessory pancreatic duct in quantities varying from 3 cc. to 10 cc. In each instance some ink escaped from the mouth of the main pancreatic duct. The

smallest quantity used (3 cc.) was injected in a large dog weighing about 15 kgm. In all cases the pancreas immediately became grossly mottled with ink escaping from ruptured acini. No haemorrhages were seen in the gross or microscopically. Microscopical sections revealed the presence of ink-particles not only in the ducts and acini, but also in the interstitial tissue between the acini in most of the pancreatic lobules. None of the larger ducts were found to be ruptured, but clumps of ink in many places obscured the minute ductules entering the acini, suggesting that some of these had been broken; and, in addition, thin lines of ink were found separating acinar cells in many places, extending from the acinar lumen to the interstitial tissue where they became merged with extravasated masses of ink. There are, normally, clefts between the acinar cells (30), and it is clear that material injected into the duct enters these clefts and may split the cells apart, permitting the injected material to escape between the separated cells.

It is evident from these experiments, which are in agreement with the observations of Mann and Giordano (15), that the quantity of bile or other fluid ordinarily injected into the pancreatic duct for the production of experimental pancreatitis in dogs (5 to 10 cc.) is more than sufficient to rupture the pancreas and to flood the interstitial tissues with the fluid injected.

Using the same method of injection with the addition of aseptic precautions, we carried out another series of experiments in which sterile Locke's solution, colored with trypan blue, was forced into the duct. The duodenal and abdominal wounds were sutured and the animals allowed to recover from the anaesthesia. Quantities of from 4 cc. to 10 cc. of the solution were injected into 8 dogs and the animals were killed with ether at intervals of from 1 to 4 days after operation. The injections immediately imparted to the pancreas a bluish mottling and an oedematous appearance. In all cases, the pancreas, except for a slight bluish color, appeared normal at autopsy. Microscopical sections showed some oedema and scattered leucocytes in the interlobular tissue at the end of 24 hours; this was even less prominent at the end of 48 hours, and had disappeared completely within 3 days. It was surprising to find that, in all of the sections, the pancreatic ducts and acini appeared intact and entirely normal. Much of the injected fluid no doubt escaped by separating the acinar cells, as observed in our first experiments. Apparently the cells are not seriously injured by this mechanical separation; in the absence of high pressure produced by duct obstruction, or of more serious damage to the acinar cells produced by the injection of more irritating substances, they apparently fall back into apposition and the defects are rapidly sealed. If ducts were ruptured by the injection, it could not be detected in the sections taken at the time of autopsy. In view of the results of later studies, it is clear that the failure of this procedure to produce more serious damage depended upon (1) the fact that the volume of the injected Locke's solution greatly diluted the small amount of

pancreatic secretion present in the ducts at the time of injection, and (2) the fact that the pathway by which the injected fluid escaped became rapidly closed, preventing a continuous leakage of pancreatic juice.

THE SPECIFIC VASCULAR LESION OF HAEMORRHAGIC PANCREATITIS

In a careful study of the pancreas in human cases of haemorrhagic pancreatitis we were struck by the constant presence of a peculiar type of vascular necrosis which had previously escaped our attention, and which we have been unable to find clearly described in text-books or autopsy reports dealing with haemorrhagic pancreatitis. This vascular lesion has the following characteristics. The adventitia of the affected artery or vein may appear condensed and pink-staining and may contain leucocytes or necrotic cells; but the striking changes are in the media. The muscle fibers of the media swell, and are sometimes separated by a pink-staining fluid or by spaces in which nothing stains; their nuclei become shrunken, pyknotic and often karyorrhectic, and polymorphonuclear leucocytes may be found in the lesions in the early stage. The internal elastic lamina, as seen in Verhoeff stains, loses its undulations and takes on a swollen appearance. Individual fibrils split off, so that the elastic membrane appears frayed. Breaks appear in the membrane, or it ceases to take the stain, as though it were completely dissolved. Finally, all nuclear staining is lost, and the necrotic tissue of the vessel wall stains homogeneously pink with eosin-haematoxylin, and frequently melts into a pink-staining or sometimes bluish, hyaline mass. The first alterations in the media are always found in the outer layer, the muscle fibers of which may be necrotic while those near the intima remain intact; but the damage in most cases proceeds rapidly to involve the entire thickness of the vessel wall with final destruction of the intima. The lesion may be localized to a segment of the vessel wall or it may involve the entire circumference (figs. 1, 2, 3). Complete necrosis and rupture of the wall of an artery may occur in a small segment of its circumference, while the remainder maintains a perfectly normal appearance and structure. Destruction of a segment of the vessel wall was found most frequently in the larger arteries, while destruction of the whole circumference was the rule in the smallest vessels. When the smaller arteries and arterioles have become necrotic or

hyalinized, their appearance is often quite indistinguishable from that characteristic of the familiar arteriolonecrosis and hyaline arteriolosclerosis occurring in man in association with hypertension and in arteriolosclerotic nephritis. In the experimental production of the lesion described we were rather astonished to find that complete hyalinization of the walls of arterioles occurred within so short a time as 24 hours.

Thrombi were often found in the injured veins, but less frequently in the arteries. Rupture of the necrotic vessels with abundant haemorrhage was not infrequently seen. In the larger arteries, haemorrhages into the outer layers of the media were frequently observed. In one case in which serial sections were made, the medial haemorrhage was found to be the result of the dissection of the vessel wall by haemorrhage from a nearby area in which the entire thickness of a segment of the wall of the artery was necrotic.

For the purpose of comparison with our cases of haemorrhagic pancreatitis in human beings, we produced pancreatitis in one dog by injecting 4 cc. of sterile bile, and in two others, by injecting 5 cc. and 10 cc. of an active solution of commercial trypsin, into the pancreatic duct. The preparation of this solution will be described presently. The operations were performed with aseptic technique, and the injections, as before, were made with a syringe into the accessory pancreatic duct. The dog injected with 10 cc. of trypsin died 18 hours after operation. The dog injected with bile was killed with ether, 24 hours after operation, and the third dog after an interval of 48 hours. In each instance, typical haemorrhagic pancreatitis was found at autopsy. This, of course, is in accord with the observations of many previous investigators. Haemorrhages in the pancreas were more abundant and widespread in the dogs injected with trypsin than in the one injected with bile, but no other essential difference could be detected in the character of the gross lesions. Typical necroses in the pancreatic tissue, with fat necroses and haemorrhages, were seen microscopically. Special stains to demonstrate bacteria were made on representative sections, but no bacteria could be found.

The examination of the microscopical sections of the pancreas, from these cases of experimental pancreatitis, showed precisely the same

peculiar vascular necrosis as that which we found in the human cases (figs. 4, 5). Aside from a paper published 25 years ago by Rosenbach (21), who mentioned briefly the occurrence of necrosis of blood vessels in the dog's pancreas into which suspensions of streptococci or bile mixed with commercial trypsin had been injected, we have not been able to find any description of vascular changes in either experimental or human pancreatitis at all suggestive of the lesions which we have described, except for a paper by Löwenthal (47) in which he described medial necrosis of small arteries in the pancreas in human pancreatitis. The lesion observed by Löwenthal must certainly have been of the same type as that here described, although he states that the elastic fibers throughout the affected vessels were always perfectly intact and that the intima was never affected. He never observed rupture of the affected vessels and did not relate the haemorrhage to such an event. The cause of the vascular lesion "whether mechanical, endogenous or exogenous toxic, or of a reflex nature" was undetermined.

The recognition of the constant occurrence of this peculiar and rapid necrosis of the walls of the pancreatic vessels, which we have found with regularity in all of our cases of haemorrhagic pancreatitis, whether in the human being or in the experimental animal, removes all mystery from the question of the immediate cause of the extensive haemorrhage that occurs in this disease. It is perfectly clear that some substance capable of exerting a powerful necrotizing action upon the walls of arteries and veins is liberated into the interacinar tissue during the development of haemorrhagic pancreatitis, and that the haemorrhage which occurs results from the rupture of the necrotic blood vessels. Since the vascular lesions were readily found in all of our human and experimental cases, regardless of the nature of the immediate inciting cause of the pancreatic damage, it seemed reasonable to believe that they were caused by some substance liberated by the pancreas itself, and we therefore turned to an examination of the possibility that trypsin might have the power to effect this specific form of vascular damage. As stated in the introduction to this paper, it has been frequently suggested that trypsin may have the ability to destroy the walls of arteries and veins because of its "corrosive power," but up to the present there has been no direct evidence that

this is really the case. Dragstedt and his collaborators, in their recent paper on haemorrhagic pancreatitis, state that "it is probable that trypsin has no corrosive properties in itself which would injure living cells" (18).

THE EFFECT OF TRYPSIN UPON BLOOD VESSELS

Commercial Pancreatic Trypsin. In order to determine whether trypsin exerts a necrotizing effect upon arteries and veins of the nature of that which we have described as occurring characteristically in haemorrhagic pancreatitis, we engaged in a study of the action of trypsin upon blood vessels in a site removed from the complicating factors which are present in the substance of the pancreas. To this end we injected solutions of commercial pancreatic trypsin into the subcutaneous tissue of dogs.

The trypsin, made from beef pancreas, was supplied in the form of a powder which, according to the statement of the manufacturers,[1] was free from lipase and diastase and contained no preservative or other added substance. One gram of the powder was added to 20 cc. of normal saline, the mixture shaken for 15 minutes and then centrifugalized for 10 minutes. The supernatant fluid was pipetted off from the insoluble sediment and passed through a Berkefeld filter for immediate use. This solution, sterile on culture, dissolved fibrin rapidly in the incubator. A solution prepared in this way was used for the production of experimental pancreatitis in the two dogs already mentioned.

Subcutaneous injections of 1 cc. of the trypsin solution were made in several places on the abdomen of each of 5 dogs. The skin and subcutaneous tissue at the sites of injection were removed and examined at the end of 24 hours. In all cases marked haemorrhage and oedema were found in the subcutaneous tissue at the site. No fat necroses were seen in the gross or microscopically. Microscopical sections showed the constant presence of vascular lesions identical in all respects with those which we had found in the pancreas in human and experimental haemorrhagic pancreatitis (fig. 6). The subcutaneous tissue was oedematous and contained numerous haemorrhages. There were areas of necrosis and liquefaction of tissue, some of which were filled with partly decolorized red blood cells together with scattered polymorphonuclear leucocytes, the latter being

[1] Fairchild Bros. and Foster, Washington and Laight Streets, New York City.

much more numerous around the margins of the necroses. Sections stained to demonstrate bacteria revealed none. Experiments in which the standard solution of trypsin was diluted with normal saline showed that the power of the trypsin, in this particular solution, to produce the vascular lesions was lost at a dilution of 1 volume in 16.

Control injections of the trypsin solution, after it had been heated in boiling water for 10 minutes, produced only very slight oedema of the subcutaneous tissue without haemorrhages. No lesions in the arteries or veins could be found in microscopical sections.

Purified Crystalline Pancreatic Trypsin. Through the kindness of Dr. J. H. Northrop, of The Rockefeller Institute at Princeton, N. J., we obtained samples of crystalline pancreatic trypsin and crystalline pancreatic chymo-trypsin, the preparation and properties of which have been described by Northrop and Kunitz in recent publications (25, 26). Solutions of these preparations were made, according to instructions, by dissolving each in 100 times its weight of N/200 hydrochloric acid. Part of each solution was passed through a Berkefeld filter and injections of the sterile filtrates, as well as of the unfiltered solutions, were made. In addition, equal parts of the trypsin and chymo-trypsin solutions were mixed and injected together.

The three different solutions were injected in quantities of 1 cc. into the subcutaneous tissue of dogs. At the end of 24 hours, the skin and subcutaneous tissue at the sites of all injections showed marked oedema and extensive gross haemorrhages. No fat necroses were present. The most extreme changes were produced by the solution of crystalline trypsin. In all cases, the sterile Berkefeld filtrates were rather less destructive than the unfiltered solutions, presumably because of the adsorption of some of the enzyme by the filters, but in all cases microscopical sections revealed the presence of the characteristic lesions in the walls of the arteries and veins, identical in all respects with those produced by commercial trypsin and with those observed in haemorrhagic pancreatitis (fig. 7).

The dry samples of crystalline trypsin and chymo-trypsin contained 50 per cent of magnesium sulphate. Control injections of 1 cc. of a solution of 0.5 gram of magnesium sulphate in 100 cc. of N/200 hydrochloric acid resulted in oedema of the subcutaneous tissue at the sites of injection, but they produced no haemorrhages, and no lesions in the vessel walls could be found microscopically.

Vegetable Trypsin. In order to determine whether tryptic ferment of plant origin exerts an effect upon blood vessels similar to that caused by pancreatic trypsin, experiments were carried out in which solutions of papain were injected subcutaneously. These experiments were undertaken not because of any direct relation to haemorrhagic pancreatitis, but because of certain considerations, to be discussed elsewhere, arising from the histological identity of some of the vascular lesions produced by trypsin with those of human hyaline arteriosclerosis.

Papain is a well-known trypsin-like enzyme (27) obtained from the fruit of the pawpaw tree (Carica papaya). Because of its ready availability it was used as an example of a tryptic ferment of plant origin. The solutions of papain (Merck) were prepared by adding 1 gram or 2 grams of the powder to 20 cc. of normal saline. The mixture was shaken for 15 minutes and then centrifugalized for 10 to 30 minutes. The supernatant fluid was pipetted off from the insoluble residue, passed through a Berkefeld filter and used immediately. Subcutaneous injections of 1 cc. of either strength of solution produced in dogs extensive haemorrhages and necroses at the sites of injection. Vascular lesions identical with those produced by pancreatic trypsin were found microscopically.

Pancreatic Duct Juice. The experiments described above demonstrated clearly that sterile, active pancreatic trypsin has the power to destroy the walls of previously normal arteries and veins in the absence of the other pancreatic enzymes, and that the vascular damage produced is histologically identical with that regularly found in the pancreatic vessels in human and experimental haemorrhagic pancreatitis.

Further experiments were undertaken, therefore, to determine whether the pancreatic secretion, as it exists in the pancreatic ducts before entering the duodenum, has the ability to produce the vascular lesions described if it escapes into the tissues. For this purpose, the pancreatic juice of dogs was collected by cannulating the accessory pancreatic duct outside the duodenum in order to avoid any contamination with enterokinase.

Using aseptic technique, the abdomen was opened in the mid-line and the duodenum carefully drawn up into the wound. The accessory pancreatic duct

was exposed by gently separating the adherent lobules of the pancreas from the duodenum in the region of the duct. A ligature was placed about the duct and tied tightly near the duodenum. The duct was then opened and a glass cannula, inserted toward the pancreas, was tied in place. The operation was performed as rapidly as possible, and the dogs were kept under light ether anaesthesia while the juice was being collected. As soon as 1 to 2 cc. of secretion had accumulated in a sterile container, it was taken up in a sterile syringe and part of it immediately injected into the subcutaneous tissue of another dog. In each experiment, part of the juice collected was cultured, and in each case it proved to be sterile. The skin and subcutaneous tissue at the sites of injection were removed and examined at the end of 24 hours.

In four experiments, quantities of from 0.5 cc. to 1 cc. of pancreatic duct juice obtained in this manner were injected. Fat necroses were produced at the sites of injection, but in none of these experiments were we successful in producing the characteristic vascular lesions with their accompanying haemorrhages. In the collection of these samples of juice, we had fed fasting dogs immediately before the operation, and injected secretin or pilocarpine after cannulation of the duct. Since it has been shown that the pancreatic secretion reaches its height in abundance and activity two or three hours after a meal, and that even the secretion excited by pilocarpin is less potent after a period of fasting than at the height of digestion, the procedure adopted in the above experiments was not calculated to yield a highly potent juice. In four subsequent experiments, therefore, we collected the pancreatic juice from dogs which had been starved for 36 to 48 hours and then fed a large meal of meat and milk 2 to 3 hours before operation. These samples of pancreatic juice, collected without injecting pilocarpin or secretin, were distinctly more viscid than those previously obtained and proved to be much more potent. Subcutaneous injections of 0.3 cc., 0.7 cc., and 0.8 cc. of the respective juices produced not only fat necroses but also the characteristic "trypsin" necrosis of the walls of arteries and veins with haemorrhages (fig. 8, 9, 10). The fluid injected was in each case sterile on culture, and no bacteria could be stained in the microscopical sections. The fourth juice obtained in this manner produced in the subcutaneous tissue of a dog much less fat necrosis and haemorrhage than the preceding three juices, and only an occasional artery was found with a necrotic wall.

In one experiment we made a pancreatic fistula in a dog by tying a short cannula in the accessory pancreatic duct outside the duodenum, and connecting it by a rubber tube with a rubber balloon fastened on the abdomen. The abundant sterile juice collected in this way was, even after the dog had started to take food following operation, thin and watery, with only a barely discernible opalescence. Several samples of this juice, activated by the addition of juice scraped from the duodenal mucosa of another dog, showed only a low degree of proteolytic activity *in vitro*. Subcutaneous injections of the unactivated, sterile, pancreatic juice failed to produce any appreciable alteration of the blood vessels in the area. Perhaps if the fistula had remained uninfected for a longer time, a more potent secretion might have been obtained, but on the fourth day, alpha hemolytic streptococci were found in pure culture in the fistula juice, and the dog died from peritonitis on the day following. It is important to note, in relation to the general problem before us, that the pancreatic ducts were dilated at autopsy, and that in microscopical sections they were found to contain many Gram-positive cocci and various kinds of bacilli, but there was no haemorrhagic pancreatitis. There were small fat necroses about the pancreas, but no vascular necrosis or haemorrhage. The subcutaneous injections of the juice demonstrated that it lacked the power to destroy the walls of blood vessels. The experiment shows that infection of the ducts is not sufficient to cause haemorrhagic pancreatitis in the absence of juice of potent tryptic power.

In 3 experiments we injected 1 to 2 cc. of sterile gall-bladder bile from dogs into the subcutaneous tissue of other dogs. At the end of 24 hours, the sites of injection showed moderate subcutaneous oedema but no haemorrhages. In microscopical sections, no vascular lesions similar to those produced by trypsin were to be found. The walls of some of the small arteries were slightly oedematous, but there was no necrosis of cells in the vessel walls.

THE PROTEOLYTIC AND NECROTIZING ACTIVITY OF PANCREATIC DUCT JUICE IN THE ABSENCE OF ENTEROKINASE

It is evident from the results of the experiments described above that pancreatic juice collected from the pancreatic duct before contact with the duodenal contents possesses properties which vary greatly depending upon the conditions under which the juice is secreted. We have observed gross differences in the physical properties of the juice, and have corroborated the many previous observations that the concentration of its enzymes is subject to great variations. The pancreatic secretion collected by means of a permanent fistula

may be so dilute as to possess only negligible necrotizing activity, while that obtained in a short experiment may attain great potency, especially during the digestion of a large meal, rich in proteins.

The fact that pancreatic juice, as it exists in the pancreatic duct, can produce the vascular lesions which we have attributed to pancreatic trypsin requires explanation. It is usually stated that the proteolytic enzyme of the pancreatic juice is secreted, under ordinary conditions, in the form of an inactive pro-enzyme, trypsinogen, which is activated upon entering the duodenum by enterokinase contained in the succus entericus, and that normal pancreatic secretion prior to activation possesses only a slight or no proteolytic activity *in vitro*. But, by introducing into the tissues pancreatic duct juice that had never been in contact with enterokinase, we have obtained lesions identical with those produced characteristically by active trypsin. There are two possible explanations of this fact; (1) that the pancreas, under certain conditions, secretes active trypsin; or (2) that the trypsinogen of the pancreatic juice becomes activated and acquires proteolytic potency when it escapes into the tissues.

Regarding the first possibility, Bayliss (29), Babkin (28), Savitsch (32) and others state that the pancreatic secretion induced by vagus stimulation contains active trypsin. If this be true, if the juice that escapes into the tissues happens to be juice that was secreted under the influence of vagus activity, it may possess tryptic activity independent of any extrapancreatic activation.

But apart from the possibility of a secretion of active trypsin, it is important that trypsinogen can be converted into active trypsin by means other than contact with enterokinase; and there are several circumstances which could explain the activation of trypsinogen when it escapes into the tissue spaces. In the first place, neutralization of the alkaline pancreatic juice accelerates spontaneous activation *in vitro* (31), and its neutralization by the buffers of the tissue fluids may be expected to have the same effect *in vivo*. Furthermore, the trypsinogen of pancreatic juice is converted into active trypsin on contact with calcium ions (27). When added to pancreatic juice *in vitro* the greater part of the calcium serves merely to precipitate carbonates and other salts, thus neutralizing the juice, while the part which really activates the trypsinogen is extremely small, so that pancreatic juice, especially if previously neutralized, is highly sensitive to the activating effect of calcium ions (27). It seems reasonable, therefore, to assume that the calcium ions present in the tissue fluids can initiate the activation of pancreatic trypsinogen in this way. Normal pancreatic juice contains only infinitesimal traces of calcium, detectable by spectral analysis; but, under certain conditions larger amounts of calcium have been found in the juice, and under these conditions

the juice taken from the duct has been active proteolytically (27). Amino acids are also said to exert an activating effect upon trypsinogen.

In our experiments in which the pancreatic duct juice displayed the properties of active trypsin when introduced into living tissues, necrosis of tissue soon occurred as a result of the action of the lipase of the juice, and it is probable that contact with the necrotic tissue, although not necessary for the initial activation of the juice, at least promoted the further activation of the trypsinogen, for there is evidence that necrotic tissue may exert an activating effect upon trypsinogen. This is indicated by the familiar rapid self-digestion of the pancreas after death, and also by the fact that the proteolytic activity of finely ground, fresh pancreas increases in potency on standing (27).

Thus, apart from the fact that the pancreatic juice that escapes into the tissues may contain active trypsin, its trypsinogen may become activated when it reaches the tissue spaces, and this without any participation of enterokinase.

Whatever may be the exact mechanism of activation, it is clear from the results of the above experiments that pancreatic juice, when it escapes from the normal channels of the gland into the interstitial tissue, can exert a potent necrotizing action upon blood vessels, producing the specific lesions that are characteristic of the action of purified trypsin, and leading to the occurrence of haemorrhagic pancreatitis. We have found no correlation between the power of a sample of duct juice to produce fat necrosis and its power to produce vascular necrosis. Extreme fat necrosis was produced by some juices in the absence of vascular necrosis, when injected into the subcutaneous tissue. The lipase, therefore, is not responsible for the vascular necrosis. Trypsin, on the other hand, as shown above, produces vascular necrosis in the complete absence of fat necrosis. Whether or not haemorrhagic pancreatitis will develop following the escape of the juice will depend, therefore, upon the concentration of tryptic enzyme, and upon whether or not the juice comes into contact with blood vessels of appreciable size. We shall discuss presently the circumstances which lead to the escape of the juice into the interstitial tissue.

It is not necessary to assume that the widespread death of the acinar cells of the pancreas in haemorrhagic pancreatitis is caused by the direct action of the juice that has escaped. Indeed, if the observation that the acinar cells are able to secrete active trypsin (32) is

correct, it is obvious that, unless these cells were resistant to the action of the ferment, they would be destroyed by their own secretion. There is, however, still uncertainty regarding the concentration of trypsin that may be secreted in active form; and it is possible that, although the living pancreatic tissue is resistant to the concentrations of active trypsin that it secretes, it may be injured by higher concentrations of activated trypsinogen. Dragstedt and his collaborators (18), in their recent publication, report experiments in which a portion of the living pancreas was implanted in the lumen of the duodenum without disturbing the blood supply of the part of the gland exposed to the duodenal juice that contained active trypsin. Since the implanted portion of the gland is said to have resisted digestion by the duodenal trypsin, they concluded that the pancreas is resistant to the action of trypsin, and that even if trypsinogen were activated within the duct system, acute pancreatic necrosis would not result. Apart from the fact that the illustrations suggest strongly that the surface of the gland that was in contact with the duodenal juice was destroyed and replaced by scar tissue (a result regularly noted by Rosenbach (21) in experiments of precisely the same type carried out some years ago) there are numerous objections that may be raised to conclusions drawn from this type of experiment. It may be remarked that these investigators accept the view that bile exerts a potent necrotizing effect upon the pancreas, stating that "there seems to be little doubt but that the pancreas is more susceptible to the local necrotizing effect of bile or bile salts than most other tissues." Since they state that the portion of the pancreas implanted in the duodenum below the ampulla remained unharmed, even though it obviously was exposed to the duodenal bile, they apparently overlooked the fact that the experiment would argue equally well against the view that the pancreas can be injured by bile.

In any event, it is clear, and important to recognize, that in haemorrhagic pancreatitis much of the destruction of the parenchyma results from disturbance of the circulation rather than from the action of trypsin, for the necrosis of the vessel walls leads to the interruption of the circulation by rupture and thrombosis. In addition, destruction due to mechanical tearing of the gland by the arterial haemorrhage is obvious in any well developed case. The destructive effects

of the circulatory obstructions and mechanical tearing tend to open up still further portions of the duct system, permitting the escape of still more pancreatic juice and favoring the development of still further thrombosis; and the process, beginning at one point, may thus spread progressively throughout a large area of the gland.

We have shown above that some samples of juice will produce only fat necrosis when injected into the subcutaneous tissue, while other samples produce both fat necrosis and vascular necrosis with haemorrhage; and that the power of the juice to produce vascular necrosis depends upon its trypsin content. If the pancreatic juice escapes into adipose tissue containing no large vessels, or if the juice has a very low trypsin content, fat necroses alone will result, and this is a quite common occurrence. The escape of pancreatic juice from the acini at the margin of the gland may cause necrosis of the walls of small blood vessels only, with the production of localized extrapancreatic haemorrhage associated with fat necrosis, and we have such cases in our material. Localized or widespread haemorrhagic pancreatitis will be produced by escaping juice only if the juice possesses sufficient tryptic activity and if, when it escapes, it comes into contact with blood vessels of some size in the substance of the gland.

To what extent variations in the antitryptic power of the blood plasma and tissue fluids may play a rôle in influencing the action of the escaping tryptic ferment we cannot say at present; but since marked variations in the antitryptic power of the plasma are known to occur under various conditions (49), it is not impossible that this factor may be important in some cases. If, for example, a juice with a relatively weak, but still effective, tryptic activity escaped in a body in which the antitryptic power of the plasma were at a very high level, the necrotizing tendency of the juice might be completely neutralized; whereas if the antitryptic activity of the plasma were low, the damaging power of the tryptic ferment would not be inhibited. These relations, however, require further study before antitrypsin can be assigned any definite rôle in the pathogenesis of haemorrhagic pancreatitis.

THE PATHOLOGICAL BASIS FOR THE ESCAPE OF PANCREATIC JUICE INTO THE INTERSTITIAL TISSUE OF THE PANCREAS

Since we have seen that the specific vascular necrosis responsible for the haemorrhage in haemorrhagic pancreatitis is caused by the tryptic ferment of the pancreatic juice, it becomes necessary to inquire

into the cause of the escape of the juice from the normal channels into the interstitial tissue where the arteries and veins are situated.

The escape of pancreatic juice into the interstitial tissue of the pancreas obviously can occur only through destruction or rupture of ducts or acini. A number of ways in which this may come about may be listed: (1) external trauma; (2) infarction; (3) infection with destruction of tissue; (4) retrojection of bile into the pancreatic duct with rupture of the acini; and (5) pressure due to obstruction of ducts outside the pancreas or within its substance.

All are agreed that *trauma* is a rare cause of haemorrhagic pancreatitis.

Infarction, leading to disruption of the channels through necrosis of tissue undoubtedly occurs (*e.g.*, autopsy 10645, table I), but this, too, is infrequent. It must be remembered that thrombi quite regularly form in many of the vessels the walls of which are damaged by the tryptic ferment, so that the mere presence of thrombi is by no means proof that the damage to the pancreas was produced primarily by thrombosis. Thrombosis should never be regarded as the primary cause of the damage unless the evidence that the vascular occlusion was primary is perfectly plain, and unless the existence of all other conditions which can permit the escape of pancreatic juice has been eliminated.

Scarring of the pancreas is not infrequently encountered in association with severe arterio- or arteriolosclerosis. In these conditions the local reduction of the blood supply responsible for the loss of tissue ordinarily occurs gradually, and atrophy, leading to final disappearance of foci of parenchymal tissue, the place of which is then taken by scars, is the rule. It is as unusual for sudden necrosis through infarction to occur as a result of vascular sclerosis in the pancreas as it is in the kidney. But that a focus of infarction may occasionally be produced in this manner and lead to haemorrhagic pancreatitis as a result of the escape of the secretion through the portion of the system opened up by the focal necrosis need not be doubted. Atrophy and gradual loss of portions of the pancreatic parenchyma occur also in severe chronic passive congestion. Here, too, it is conceivable that an acceleration of this process may occasionally lead to a more rapid necrosis with escape of secretion. In all such cases, however,

TABLE I

Twenty-four cases of acute haemorrhagic pancreatitis

AUTOPSY NUMBER	AGE SEX COLOR	OBSTRUCTION EVIDENCED BY DILATED DUCTULES OR ACINI	OBSTRUCTING METAPLASIA OF DUCT EPITHELIUM IN AVAILABLE SECTIONS	OBSTRUCTION OF MAIN PANCREATIC DUCT	BILIARY TRACT DISEASE	OBESITY	ALCOHOLISM	PREVIOUS DIABETES	EXTENT OF PANCREATIC DAMAGE
13170	37, F. W.	+	+	0	0	+	+	0	Diffuse
13103	13, F. W.	+	+	0	0	0	0	No data	Diffuse
12632	62, M. W.	+	+	0	Stones in gall-bladder	0	0	0	Diffuse
12244	64, M. W.	+	+	+ Duodenal diverticulum in head of pancreas	Stones in gall-bladder	0	0	0	Diffuse
11783	59, M. W.	+	+	0	0	0	0	0	Focal
10593	13, F. W.	+	+	0	0	0	0	+	Diffuse
2855	13, F. W.	+	+	0	0	+	0	No data	Diffuse
2860	65, F. W.	+	+	0 Duct opens separately from bile duct	Stones in gall-bladder	0	No data	0	Diffuse
2394	37, M. W.	+	+	0	0	0	0	0	Focal
S.H. 457	62, F. W.	+	+	0	0	0	No data	No data	Diffuse
B.V. 3507	34, F. W.	+	+	0	0	0	+	0	Diffuse
B.V. 6283	42, M. W.	+	+	0	0	0	+	0	Diffuse
10176	52, F. W.	sl.	+	0 Duct opens separately from bile duct	Stones in gall-bladder	0	No data	0	Diffuse
11248	20, F. C.	sl.	0	0	0	0	+	+	Focal
11675	32, F. C.	sl.	0	0	0	+	+	0	Diffuse

1567	47, M. W.	+	0	+ Compressed by stone in bile duct 1.5 cm. above ampulla. No bile in pancreatic duct	Gall-stones	0	0	0	Diffuse
1691*	40, M. W.	+	0	+ Stone impacted in ampulla into which bile and pancreatic ducts open*	Gall-stones	+	0	No data	Diffuse
2664†	51, M. W.	+	0	+ Stone impacted in ampulla into which bile and pancreatic ducts open†	Gall-stones	0	No data	No data	Diffuse
11227	28, M. W.	+	0	0	0	0	+	0	Focal
12761	58, F. C.	+	0	0	0	0	0	0	Focal
13906	40, M. W.	+	0	0	0	+	+	0	Diffuse
14445	32, M. C.	+	0	0	0	0	No data	0	Diffuse
B.S. 89	41, M. W.	+	0	0	0	+	No data	No data	Diffuse
10645	15 mos. F. W.	0	0	0	0	0	0	0	Focal infarction

* Opie's original case with stone in the ampulla, reported from this Department in 1901 (reference 8).

† Reported by Bunting from this Department in 1906 (reference 45).

it seems likely that, unless an obstruction to the outflow of secretion through the duct is present, the secretion will not be forced in any quantity into the interstitial tissue through such focal disruptions of the parenchyma. Experimentally, we have found it impossible to produce haemorrhagic pancreatitis by destroying traumatically minute areas of a normal pancreas.

The idea that *bacterial infection* is a common cause of haemorrhagic pancreatitis is certainly unproved at present. It is true that cholelithiasis is not uncommonly associated with haemorrhagic pancreatitis (in slightly less than one-third of our cases in table I), and that focal pancreatic scarring (so-called "chronic pancreatitis") is not uncommon in association with cholelithiasis; but this does not warrant the assumption that the loss of pancreatic tissue was a result of bacterial infection of the gland extending from the bile passages. In two of the cases in table I, in which stones were found in the gall-bladder in association with haemorrhagic pancreatitis (Nos. 2860 and 10176), the pancreatic duct opened into the duodenum separately from the bile duct. Had the dissection not been made, these cases would have been placed by many in the group assumed to be caused by spread of infection from the bile passages to the pancreatic duct. As a matter of fact, there was no active infection of the bile passages in these cases. Indeed, the presence of stones in the gall-bladder constitutes the only evidence of active infection in the majority of cases in which haemorrhagic pancreatitis associated with gall-stones has been assumed to be due to infection. It is hardly necessary to point out that the presence of stones in the gall-bladder is in no sense evidence that active infection of the biliary tract exists. It is well known that stones are found in the gall-bladder at autopsy in approximately 10 per cent of all adults. In regard to the association of pancreatic scarring with cholelithiasis, it must be remembered that in cases in which the bile duct and pancreatic duct open together into the duodenum, a gall-stone lodging at or near the ampulla can readily compress the pancreatic duct and lead to loss of tissue and scarring of the pancreas as a result of obstruction in the complete absence of infection. The same result can be readily produced experimentally by obstructing the pancreatic duct, as was shown long ago by MacCallum (50); and it is our opinion that the great majority of instances

of pancreatic scarring associated with gall-stones result from obstruction, and not from bacterial infection of the pancreatic tissue. Certainly, it is exceedingly rare to encounter active bacterial infection of the pancreatic tissue; and in the great majority of cases of pancreatic scarring associated with cholelithiasis the pancreatic ducts show no evidence of infection, and the gland no more inflammation than that which is always associated with scar formation when tissue is being destroyed in the absence of infection. Dilatation of ductules and acini, indicating the existence of obstruction, is, however, almost invariably found in such cases. Furthermore, a precisely similar focal loss of pancreatic tissue with scarring is encountered with great frequency in the absence of cholelithiasis or biliary infection in association with non-inflammatory obstruction of ductules within the pancreas as we shall see in a moment. We are, therefore, unable to regard focal scarring of the pancreas as satisfactory evidence of bacterial infection.

That infection may spread from the bile duct to the pancreatic duct in certain cases is undoubtedly possible, though it has been assumed to occur far more frequently than it has been demonstrated. But even in the cases in which the infection spreads to the pancreatic duct, the infection, in itself, is insufficient to produce haemorrhagic pancreatitis. Unless it produces a disruption of the pancreatic tissue, and unless juice with potent tryptic activity escapes into the interstitial tissue about arteries of some size, haemorrhagic pancreatitis will not occur. Experimentally, infection of the gall-bladder has never been shown to produce haemorrhagic pancreatitis except in the presence of a communication between the bile duct and the pancreatic duct, together with an obstruction of the ampulla of Vater; and even under these conditions, with the bile heavily infected by the injection of cultures of streptococci, staphylococci and intestinal bacteria into the gall-bladder, Nordmann (19) was never able to produce extensive haemorrhagic pancreatitis such as that which occurs in the human being, or in the experimental animal into the pancreatic duct of which bile is injected in amounts sufficient to rupture the duct-acinar system. It is noteworthy that Nordmann's experiments were acute, and that in no instance did he find any appreciable dilatation of the pancreatic duct, even though it was bile-stained. It will be recalled

that, in one of our experiments described above, even though the pancreatic duct was dilated and heavily infected, haemorrhagic pancreatitis did not develop; and in that experiment it was shown that the juice secreted did not have a tryptic potency sufficient to cause necrosis of the walls of blood vessels.

Finally, it may be mentioned that the finding of bacteria on culture of the pancreas in cases of haemorrhagic pancreatitis is not sufficient proof that the bacteria reached the pancreas before necrosis of the tissue and rupture of the ducts had lowered the forward pressure within the system and allowed their entrance through the duct. Certainly, bacteria can rarely be stained in necrotic or normal portions of the gland in haemorrhagic pancreatitis.

For the above reasons, it is our opinion that spread of infection from the bile duct to the pancreas producing destruction of the gland is seldom a factor in the production of haemorrhagic pancreatitis. Certainly, the great majority of our own cases of haemorrhagic pancreatitis occurred in the absence of infection of the biliary tract (table I).

Regarding the *retrojection of bile* into the pancreatic duct as a cause of haemorrhagic pancreatitis, we have already discussed this possibility in the introduction of this paper. We do not doubt that, if a stone in the ampulla converts the bile and pancreatic ducts into a common channel, the contraction of the gall-bladder may force bile into the pancreatic duct, and that the acini may be ruptured by the pressure and the acinar cells damaged by the irritant action of the bile. However, we believe that when haemorrhagic pancreatitis develops under these conditions the massive haemorrhage and destruction of tissue is not a direct result of the presence of bile in the gland, but rather the result of rupture of the system permitting the escape of the pancreatic juice; for in the instances in which haemorrhagic pancreatitis occurs in association with a stone in the ampulla (table I), the pancreatic vessels show the type of necrosis that we have found to be characteristic of the action of trypsin. We have stated above that bile alone, when injected into the subcutaneous tissue, did not produce this vascular necrosis, but, when injected into the pancreatic duct, the "trypsin" vascular lesion occurred in the pancreatic interstitial tissue. It has been noted by other investigators that bile

injected into tissues other than the pancreas fails to produce the extreme haemorrhage and necrosis that occur when it is injected into the pancreatic duct. Dragstedt and his collaborators (18), for example, state that in their experiments in which bile was injected into the pelvis of the kidney "in no case could the extent of the pathologic change be compared with that resulting from the injection of a like amount of bile into the pancreatic duct." They concluded, as quoted above, that the pancreas is more susceptible to the necrotizing effect of bile than are most other tissues.

We believe that the fact that haemorrhagic pancreatitis results from the injection of bile into the duct is insufficient evidence that the pancreas is more susceptible to the direct irritant effects of bile than are other tissues. It must be remembered that haemorrhagic pancreatitis can be readily produced by the injection of practically any irritant into the duct (*e.g.*, weak acids, weak alkalis, bile salts, calcium chloride, formaldehyde, chromic acid, etc.). It is hardly conceivable that the pancreas is tremendously more susceptible to all these irritants than are other tissues, in which these substances produce far less damage when injected in dilutions that will produce haemorrhagic pancreatitis. The production of haemorrhagic pancreatitis by bile and other irritants, is, rather, attributable to the fact that, when they are injected into the duct, acini and ductules are ruptured, as we have shown above; and, if the cells at the sites of the ruptures are damaged by the irritant or spread further apart by inflammatory oedema, the openings will not become promptly sealed up as occurs after the injection of bland fluids; and if pancreatic juice of sufficient tryptic potency escapes and comes into contact with large arteries, vascular necrosis with resulting haemorrhage, thrombosis, tearing of the gland and infarction will produce the picture of haemorrhagic pancreatitis. If the bile is abnormal and highly irritating, due to infection, high concentration of bile salts, or low concentration of mucin (17), it may directly damage and even kill cells of the pancreas. It is of very considerable interest, however, that whereas bile salts (which have been shown (17) to constitute the irritant portion of the bile responsible for the primary damage that follows the injection of bile into the pancreatic duct) readily produce haemorrhagic pancreatitis when injected into the pancreatic duct, Sellards (39) found

that when they are injected into the duct of the salivary gland, definite tissue damage results, but "there are, however, no extensive haemorrhages such as are found in acute haemorrhagic pancreatitis." In the light of what has been shown above, it seems clear that this is due to the fact that the salivary gland does not secrete tryptic ferment, which, as our experiments have demonstrated, is the specific agent responsible for the vascular necrosis that occurs in haemorrhagic pancreatitis.

In summary, while the retrojection of bile into the pancreatic duct may produce haemorrhagic pancreatitis, the evidence instructs us that the extensive haemorrhage and necrosis are the result not of the action of the bile, but of the action upon blood vessels of trypsin which has escaped from the pancreas into the interstitial tissue. In our series of cases of haemorrhagic pancreatitis (table I) bile was found in the pancreatic duct in only 2 of the 24 cases, although this department has been particularly active in the search for this condition during the past 35 years. It should be mentioned that bile was found in the pancreatic duct in a third case not included in this series for the reason that microscopic sections were not available for study.

In regard to the view that most cases of haemorrhagic pancreatitis are caused by gall-stones or biliary tract disease, it is pertinent to state that, whereas the latter conditions are well known to be much more common in women than in men, the statistics of haemorrhagic pancreatitis appear to show that this condition occurs more frequently in men than in women. This is not shown in our small series of cases in table I, but it is in a larger series collected from the literature by Opie (43); and Barker (44) states that haemorrhagic pancreatitis occurs twice as frequently in males as in females. Some clinical series show a larger percentage of women; in some males predominate. Here the question of diagnosis enters. Autopsy statistics are more reliable in this regard, but the available material is smaller in amount. Gruber (4) has collected 94 cases from three autopsy services, and of these 58 were in males and 36 in females.

What, now, of *obstruction of the pancreatic duct system* as a cause of the escape of the ferment-containing juice? In our experience this is a very common occurrence, as will be detailed below. Obstruction of the main pancreatic duct with rupture of the system and conse-

quent haemorrhagic pancreatitis may undoubtedly be produced by a variety of causes. Cases of haemorrhagic pancreatitis associated with obstruction of the main pancreatic duct are familiar in the literature, and a wide variety of different types of obstruction have been described, including pancreatic calculi (33), tumors of the pancreas, a roving Ascaris, duodenal diverticula (autopsy 12244, table I), a gall-stone either in the lower portion of the bile duct or in the ampulla compressing the pancreatic duct but not permitting the inflow of bile (autopsy 1567, table I), etc. Indeed, a gall-stone in the ampulla converting the bile and pancreatic ducts into a common channel will itself obviously produce obstruction to the flow of pancreatic juice, and even though bile be not forced into the pancreatic duct, haemorrhagic pancreatitis may occur as a result of rupture of ductules or acini dilated behind the obstruction. It may be remarked that if a rupture of dilated, thinned out acini occurs under such circumstances, it may obviously lower the pressure in the pancreatic duct system, and bile may then enter the duct. The finding of bile in the duct at autopsy, therefore, does not provide certain evidence that the bile entered the duct before the acinar rupture responsible for the haemorrhagic pancreatitis occurred; and we repeat that the finding of bile in the pancreatic duct in a case in which a stone is lodged in the ampulla and in which pressure was exerted at autopsy upon the gall-bladder before opening the ducts, is in no sense proof that the bile was in the pancreatic duct during life.

While gross obstruction of the extra-pancreatic portion of the duct occurs, and may undoubtedly lead to haemorrhagic pancreatitis, we have found that a far more frequent form of duct obstruction is that which occurs within the pancreas as a result of *metaplasia of the duct epithelium*, of the type that has been described by Priesel (40), Baló and Ballon (35) and others without reference to haemorrhagic pancreatitis. This change consists in a localized proliferation of the duct epithelium which, in the affected region, loses its cuboidal or columnar character and assumes that of transitional or basal epithelium. The epithelial proliferation leads to the formation of masses of cells which project into the duct and partly or completely obstruct its lumen (figs. 11, 12, 13, 14, 15). This process affects principally the small branches of the duct system and has a spotty distribution.

It may be found in one ductule in one section and be completely absent in sections from other regions of the same pancreas. The ductules and acini behind the particular duct that is obstructed by the metaplastic proliferation of cells become greatly dilated (fig. 16, 17), and rupture of their thinned out walls not infrequently leads to the escape of the pancreatic juice with resulting localized inflammation and scarring or fatal haemorrhagic pancreatitis, as we shall see below. Complete or long continued partial obstruction of a duct by the nodules of metaplastic epithelium leads to atrophy and disappearance of the acini connected with that duct. The end stage of this process is a localized area from which much or all of the acinar tissue has disappeared, and is replaced by fibrous tissue containing only islands of Langerhans and dilated ductules (fig. 15). Such areas as this are commonly diagnosed "chronic pancreatitis," or "interstitial pancreatitis." In an illustration of a case described as "subchronic pancreatitis" due to acquired syphilis (no spirochaetes demonstrable) in the Henke-Lubarsch Handbuch (reference 4, p. 427), one can see a duct greatly obstructed by metaplasia in the microscopic field photographed to show loss of pancreatic parenchyma and scarring.

We have found this obstructing, metaplastic basal cell proliferation in one or more ducts in 13 of our series of 24 cases of haemorrhagic pancreatitis (figs. 13, 14, 15), and this figure might well have been higher had a larger number of sections of the pancreas been available for microscopical study in each case. This is indicated by the fact that localized obstruction of ducts, as evidenced by localized areas in which ductules and acini were dilated, was found in the great majority of our cases in the absence of obstruction of the macroscopically visible ducts (table I). In most of our cases we had for examination only one or two routine sections taken at the time of autopsy some years ago.

We have for some time been impressed by the frequency of this type of metaplasia of the pancreatic duct epithelium in our routine autopsy material, and in order to gain more precise information as to its frequency we examined microscopical sections of the pancreas from 150 consecutive autopsies on individuals 25 years of age and upward, but otherwise unselected, dying from various causes other than disease of the pancreas. The only cases omitted from the consecutive series

were those in which the pancreas was so autolyzed as to render proper study impossible. In most instances only one section of the pancreas was available for examination. Nevertheless, among the 150 cases, metaplasia of the pancreatic duct epithelium was found in 28 cases, or 18.6 per cent. This figure is rather higher than that of previous investigators. Priesel (40), for example, found metaplasia in about 10 per cent of 140 pancreases examined, and Baló and Ballon (35) in about 9 per cent of 160 pancreases; but the frequency of this form of obstructing metaplasia in routine autopsies is obvious from all three studies. Localized dilatation of terminal ductules or acini was present in 27 of the 28 cases in which duct metaplasia was found (figs. 16, 17), and in 11 additional cases in which the cause of the obstruction could not be discovered in the sections of pancreas available for examination. In 2 further cases among the 150 examined, dilatation of the duct-acinar system was found, in one case associated with a cyst in the pancreas and in another with a calculus in the pancreatic duct. Old or fresh fat necroses were found associated with acinar dilatation in 7 of the 150 pancreases examined, and in 2 of these 7 cases metaplasia of duct epithelium was present in the section showing the fat necrosis (fig. 17). Old or fresh localized haemorrhage was found in 3 of the 150 pancreases examined. In one of these 3 cases metaplasia was present in the same section; in the other two there was marked dilatation of the acini, but no metaplasia was found in the section. It is obvious that the obstruction of a duct producing acinar dilatation will often be situated at a different level from that occupied by the dilated acini, and that a section showing the one may fail to pass through the level in which the other is situated. Outside the above series of 150 cases examined, we have repeatedly found metaplasia associated with fat necrosis or focal haemorrhages in our routine autopsy material. While this obstructing metaplasia is extraordinarily common, it is, of course, not the only means by which obstruction of branches of the duct system is produced. Small concretions occasionally occur in the ducts within the pancreas, and it is obvious that a variety of other conditions, such as small adenomata or other masses within the pancreas (*e.g.*, autopsy 12244, table I) may more rarely produce obstruction.

From the above data it is evident that complete or partial obstruc-

tion of small ducts within the pancreas leading to marked dilatation of the ductules and acini behind the obstruction is a quite frequent occurrence, as is also the rupture of the dilated acini with the escape of pancreatic secretion. In the cases in which only fat necroses are found as a result of this acinar rupture, it seems clear that the pancreatic juice failed to come into contact with arteries and veins, or else that the secretion that escaped was too poor in trypsin to produce vascular necrosis and haemorrhage, just as in our experiments some juices obtained from the pancreatic duct produced fat necrosis but only slight or no vascular necrosis when injected into the subcutaneous tissue. The escape of juice producing only fat necrosis and slight vascular damage might well produce an attack of acute pancreatic pain even without the occurrence of haemorrhage. But even if haemorrhage does occur under such circumstances, recovery is possible if the process remains localized. We have observed several instances of haemorrhagic pancreatitis (*e.g.*, autopsies 12244 and 14455) in which repeated pancreatic ruptures had evidently preceded the fatal event, and had left unmistakable marks of previous tissue destruction in the form of organized fat necroses, or localized scars in and about the pancreas containing haemosiderin-laden cells and blood vessels with scarred walls and with canalized thrombi. It seems clear, therefore, that the outcome of the rupture of dilated acini in the pancreas depends upon the quantity and tryptic potency of the escaping pancreatic juice, and upon the size and number of the blood vessels with which it may chance to come into contact. It is obvious, furthermore, that the danger of rupture of damaged arteries will be greatly furthered by the presence of hypertension. In our series of 24 cases of haemorrhagic pancreatitis, the patients were known to have had hypertension previous to the attack in 4 instances.

Aside from the effect of metaplasia in producing duct obstruction, it is not impossible that some cases of haemorrhagic pancreatitis may result from the fact that the abnormal, metaplastic cells may at times be less resistant to the pancreatic secretion than is the normal epithelial lining of the duct. Certainly, one often finds the metaplastic cells projecting into the duct unharmed by the secretion (fig. 12), but in an abnormal production of abnormal cells of this type it is conceivable that the cells in some metaplastic areas might be sus-

ceptible to injury by the pancreatic secretion with which they come in contact. Should the destruction of a metaplastic focus occur, the duct wall would become exposed to the secretion, and the secretion exposed to the activating effect of the tissue fluids, and destruction of the wall with escape of secretion could occur. Furthermore, it is also conceivable that death of the superficial abnormal cells of an area of metaplasia might serve to activate the trypsinogen of the secretion and so initiate a progressive destructive process. At present, these possibilities must remain as surmises only. The only effect of metaplasia definitely known at present is the production of obstruction, with rupture of distended acini behind the obstruction.

Since it appears that pancreatic duct obstruction due to metaplasia of the duct epithelium plays an important rôle in the pathogenesis of many cases of haemorrhagic pancreatitis, it is interesting to inquire into the cause of the metaplastic modification of the epithelium. Upon this point there is, unfortunately, practically no concrete information. In our material there is no evidence whatever that inflammation of the duct plays any rôle in the production of the metaplasia. In even the very earliest stages of the process the walls of the ducts beneath the metaplastic epithelium are ordinarily quite normal in appearance. Nor is there any evidence in our material that obstruction from any other cause precedes the metaplasia, as suggested by Baló and Ballon (35). In two thirds of our cases of haemorrhagic pancreatitis in which metaplasia was found there was no infection of the biliary tract and no cholelithiasis; and in the 28 cases in which we found metaplasia in the series of adults dying from causes other than pancreatic, there were only 4 in which cholelithiasis, biliary tract infection or dilatation of the bile ducts was present. When it is remembered that approximately 10 per cent of all adults have gall-stones, it is clear that the incidence of biliary tract disease in our series of cases of metaplasia is hardly more than would be expected in any series of adults, and certainly not great enough to be regarded as playing any appreciable rôle in the development of the metaplasia.

That obstruction does not tend to produce metaplasia of this type is further suggested by the situation in an interesting series of five cases that we have recently studied. In these cases, all in young children, there was found an extreme dilatation of the ducts throughout the pancreas, with disappearance of practically all of the acinar tissue, which had been replaced by scar tissue. Clearly there must have been an obstruction of the main pancreatic duct in these cases, for the duct dilatation and loss of tissue were present even in the head close to the duodenum. Unfortunately, the precise cause of the obstruction was not determined in any of these cases, for the condition was not recognized before the gross material had been discarded. It seems possible that these may represent instances of congenital atresia of the pancreatic duct, and we hope to be able in future cases to ascertain

the cause of the obstruction by serial sections. However, in none of these cases of extreme obstruction was there metaplasia of the epithelium of any of the obstructed ducts.

The squamous metaplasia that occurs in children in vitamin A deficiency (36) has a very different appearance from the type of metaplasia under discussion, although in some cases they do resemble each other. We have a number of cases of vitamin A deficiency in our autopsy files, and, as described by Wolbach and Blackfan (36) and Wilson and Dubois (48), the metaplastic cells are more typically squamous, and keratinization, which never occurs in the metaplasia under discussion, is prominent. The metaplasia in vitamin A deficiency, furthermore, is not confined to the pancreatic epithelium, but occurs simultaneously in other sites (bronchi, bladder, ureter, renal pelvis, etc.). As a matter of fact, it is hardly conceivable that vitamin A deficiency could be so widespread among adults as is the type of metaplasia under discussion, for the adult diet contains abundant sources of vitamin A. It is interesting to remark that the squamous metaplasia of vitamin A deficiency was found in various epithelial tissues outside the pancreas in the cases mentioned above in which there was pancreatic duct obstruction in young children, but there was neither squamous nor basal cell metaplasia of the pancreatic duct epithelium. The fat soluble vitamin A deficiency in these cases was probably due to deficient fat absorption resulting from the obstruction to the external secretion of the pancreas. Blackfan and Wolbach (36) have encountered similar cases.

It is at present impossible for us to advance any really plausible suggestion as to the etiology of the common type of metaplasia not associated with vitamin A deficiency. It constitutes, however, an extraordinarily frequent and important cause of localized obstruction of the pancreatic ducts, and its etiology is a matter that deserves careful study. Of the 28 instances of this type of metaplasia found in the 150 consecutive adult autopsies mentioned above, 20 occurred in males and 8 in females. The youngest individual in which we have seen this type of metaplasia was a child 13 years of age; the oldest was a man 85 years of age. In our material outside the present study the condition has been considerably more common in adults than in children, and this has been noted by others. This fact, in itself, speaks against vitamin A deficiency as a cause of the metaplasia.

THE FREQUENCY OF DUCT OBSTRUCTION IN THE PRESENT SERIES OF CASES OF HAEMORRHAGIC PANCREATITIS

In table I it will be seen that dilatation of ductules and acini, with flattening of the lining epithelium, indicating obstruction to the

outflow of secretion, was found in 23 of the 24 cases of haemorrhagic pancreatitis in which microscopical sections were available for study. In 2 of these 23 cases (1691 and 2664) a gall-stone was found in the ampulla, and the pancreatic duct contained bile. It is safe to say that this situation was not overlooked in any of the other autopsies, for the first demonstration of this cause of haemorrhagic pancreatitis was made in this department by Opie (8) in 1901, and the condition has always been carefully searched for in subsequent autopsies. We have discussed above the question whether the bile found in the pancreatic duct in such cases actually entered the duct before rupture of the acini resulting from the obstruction had lowered the pressure in the pancreatic system; and as we have pointed out, undoubtedly at least some of the bile found in the pancreatic duct in both of these cases was forced there at the time of the autopsy by the pressure that is stated by the pathologist to have been exerted upon the gall-bladder. We have also mentioned the experimental evidence that shows that bile may enter the pancreatic duct without producing haemorrhagic pancreatitis if the acini are not ruptured by the pressure. We shall, therefore, enter no further into a discussion of the precise rôle of bile in such cases, other than to repeat that in cases in which a stone is impacted in an ampulla into which both the pancreatic duct and the bile duct empty, the pancreatic duct will obviously be obstructed, and rupture of the acini with escape of the juice into the interstitial tissue may result from the rising pressure within the system. In such cases there is, therefore, a reason for the occurrence of haemorrhagic pancreatitis even though no bile finds its way into the pancreatic duct. This is clear from the cases of haemorrhagic pancreatitis in which a stone lodges in the bile duct behind the ampulla in such a way as to press upon and occlude the pancreatic duct, and in which no bile could enter the duct (*e.g.*, autopsy 1567, table I). Undoubtedly, if bile does enter the pancreatic system when the pressure conditions are such as to cause rupture of acini, the irritant action of the bile will decidedly increase the chance that haemorrhagic pancreatitis will develop, as is clear from all experimental studies.

Excluding the above 2 cases in which bile was found in the pancreatic duct at autopsy, there remain 22 cases in our series (91.6

per cent) in which the pancreatic duct contained no bile, and in which there was no evidence of any sort that retrojection of bile played a rôle in the causation of the condition. One of these cases (10645), occurring in a 15 months old infant with a congenitally malformed heart and infarcts in the brain, spleen and kidneys was clearly the result of infarction; and there was no evidence of obstruction. In all of the remaining 21 cases there was distinct microscopic evidence of localized or widespread obstruction to the outflow of secretion (distended ducts and acini). In 14 of these 21 cases a cause for the obstruction was found. In one instance (1567) a gall-stone in the common duct compressed and occluded the adjacent pancreatic duct but did not permit bile to enter it. In one case (12244) there was obstructing metaplasia of the lining of the ductules with small concretions in dilated ductules, and in addition, pressure upon the pancreatic duct caused by a distended duodenal diverticulum embedded in the head of the pancreas. It may be remarked that the literature contains a number of similar cases in which a duodenal diverticulum was responsible for the development of haemorrhagic pancreatitis (4). In 12 other cases obstructing metaplasia of the duct epithelium was found. In the remaining 7 cases in which distended acini and ductules bore unmistakable evidence to the existence of obstruction, there were three cases (11248, 10176, 11675), to be discussed below, in which the ducts contained a greatly inspissated secretion which, itself, may have produced obstruction. In the remaining 4 cases with dilated ducts and acini, no cause for the obstruction could be determined in the material at our disposal. In none of these 7 latter cases were there gall-stones either in the gall-bladder or in the bile duct, nor was there any abnormality of the biliary tract. It is obvious that metaplasia or small concretions present at one level in the pancreatic duct system can easily be missed if the sections pass through the dilated portion of the system behind the obstruction.

In view of the relation of duct obstruction to distension of the pancreatic acini and to rupture of the acini with consequent escape of tryptic ferment that has the power to destroy the walls of blood vessels, as discussed above, the frequency with which evidence of obstruction was found in the cases of haemorrhagic pancreatitis in the present study is impressive as indicating the rôle that duct ob-

struction, whether inside or outside the pancreas, plays in the pathogenesis of the condition.

In three cases (11248, 10176, 11675) the microscopic sections show an interesting condition which suggests that the haemorrhagic pancreatitis in these cases may possibly have been due in some way to the presence of an abnormal secretion within the ducts. In these cases there are numerous ducts which contain an inspissated secretion that stains deeply and irregularly with eosin. This abnormal secretion may fill homogeneously a cross section of the duct, or it may appear as separate, coagulated masses of different sizes lying in the lumen, apparently separated from each other by thinner secretion. There may be no inflammation either in the lumen or the wall of the duct, but when inflammation is present the individual masses of inspissated secretion may be found to be separated from each other by infiltrating groups of leucocytes (fig. 18). The entire lining of some of the ducts is destroyed. In other ducts the lining is preserved around a portion of the circumference, and is destroyed in a segment only. Where the epithelium is lost, the entire thickness of the wall of the duct may be completely destroyed and liquefied. In such areas polymorphonuclear leucocytes are present in abundance, and they invade the damaged ducts in large numbers (fig. 18). Wherever the epithelial cells of a duct are lost and the inspissated secretion comes into contact with the sup-epithelial tissues, leucocytes appear to be powerfully attracted to the site, and one not infrequently sees a mass of this secretion surrounded only by a swarm of leucocytes in a site where a small duct has been completely destroyed and has disappeared. No bacteria can be stained in any of the sections. It may be recalled that sterile pancreatic-duct juice that was active in producing fat necrosis and vascular necrosis, when injected subcutaneously, called forth large numbers of leucocytes.

In one of these cases (10176) metaplasia of the epithelium of one small duct was found. In the two other cases there were no gall-stones, and no metaplasia or other obstruction was found in the material available for study. There was no appreciable dilatation of acini, but some of the ducts were dilated and their epithelium greatly flattened out.

It is perfectly clear that in these cases some of the ducts contain an abnormal secretion. We have observed a similar secretion in several other cases of pancreatitis in ducts behind obstruction, although in most cases the contents of obstructed ducts have a very different appearance, being more homogeneous, less eosinophilic, and apparently less inspissated. Although no obstruction other than the inspissated secretion itself was found in two of the three cases under discussion, it is noteworthy that the peculiar material was not secreted uniformly throughout the gland. It was present in localized groups of ducts and completely absent in many other ducts. The inspissated appearance of this abnormal secretion recalls the fact mentioned above that the highly active, necrotizing juices obtained from the pancreatic duct in our experiments were always much more viscid and glairy than were less active or inactive juices. It may also be mentioned that it has often

been shown that the highly active juice stimulated by pilocarpin is, likewise, much more viscid than less active juices, and the same is said to be true of the juice obtained by vagus stimulation in which trypsin is said to occur in active form.

Whether in these cases trypsin is secreted in active form in concentration sufficient to destroy the normal lining cells of the ducts it is impossible to say, but the fact that a duct may be destroyed on one side only, as illustrated in figure 18, indicates strongly that even this abnormal secretion is not able to destroy normal duct epithelium. Since, in all of the cases, ducts are found with greatly flattened epithelium, it is possible that cells flattened out and damaged by the rising pressure produced by the plugging of the ducts with the viscid secretion may be rendered less resistant to the irritant action of this peculiar secretion which may be unusually rich in ferment. In connection with the destruction of only a segment of the wall of a duct, it is of interest that, contrary perhaps to expectation, the cells in one segment of a duct filled with this secretion may be extremely flattened out while the cells in other portions of the circumference appear more cuboidal or almost normal.

The nature and cause of the peculiar secretion are not clear. It is clear only that when this type of secretion is present, flattening out of the epithelium of the ducts containing it may occur, and that whenever the flattened out cells die and are lost the contents of the duct tend to exert a violent corrosive action upon the underlying duct wall, and that the secretion escaping from the liquefied ducts is highly active in producing fat necrosis and vascular necrosis.

CORRELATION OF THE RESULTS OF THE PRESENT STUDY WITH CERTAIN CLINICAL OBSERVATIONS

The observations recorded in this paper, and our interpretation of them, are consistent with, and may render understandable, the clinical observation that haemorrhagic pancreatitis commonly has its onset shortly after the patient has eaten a large meal. This is a time when the rupture of ductules and acini, previously dilated from pressure within an obstructed system, would be most likely to occur; for the requirements of digestion demand a copious flow of pancreatic secretion rich in enzymes, and it is well known that a large meal greatly stimulates the secretion of ferment-rich pancreatic juice. The increased secretion will obviously raise the pressure in the ducts and acini behind an obstruction and so favor the rupture of the dilated, thinned-out ductules and acini, and the consequent escape of the juice into the interstitial tissue. The well known frequency with which the victims of haemorrhagic pancreatitis are obese individuals may very well be associated with the tendency of obese persons to eat large meals.

Attention has long been drawn to the fact that a considerable

proportion of patients with haemorrhagic pancreatitis are chronic alcoholics, or that the attack followed a bout of heavy drinking. In table I, it will be seen that in 7 of the 19 cases in which information was available, the patients were known to have been alcoholics, and there are other cases in our files in which localized fat necroses, associated with duct obstruction caused by metaplasia, have been found in chronic alcoholics (*e.g.*, autopsy 12752, fig. 12). It is, therefore, pertinent that the ingestion of alcohol has been shown to produce a marked increase in the flow of pancreatic juice (37) (46).

It is apparent from these considerations that conditions which have been long recognized clinically to be commonly associated with the onset of haemorrhagic pancreatitis (the ingestion of a large meal or of alcohol) are conditions which will raise the pressure in the duct and acinar system behind an obstruction by increasing the secretion; and that such conditions thereby favor the rupture of the distended system and the escape of the contained juice.

In this connection, it is of interest that Babkin (41) has recently confirmed previous observations that an elevation of the blood sugar causes an increased flow of ferment-rich juice. If this be the case, the intravenous administration of glucose to patients with symptoms of haemorrhagic pancreatitis, as commonly practiced, might possibly increase the damage by stimulating the further flow of juice into the interstitial tissue. It is interesting that one of the cases in table I (autopsy 13103) was a girl with obstruction of ducts within the pancreas due to metaplasia (fig. 14). She was accidently burned while in excellent health, was brought to the hospital and given a large amount of 5 per cent glucose intravenously. She died 12 hours after the glucose infusion was begun, and haemorrhagic pancreatitis was found at autopsy. While the glucose infusion may have had nothing to do with the development of the haemorrhagic pancreatitis in this case, the coincidence is worth recording in the present connection. The question may be raised whether the high blood sugar of diabetics tends to increase the flow of pancreatic juice. There is no adequate information on this point at present. It will be noted that in 2 of 19 cases of haemorrhagic pancreatitis in table I in which previous information was available the patients were known to have been diabetics.

We have stated above that rupture of dilated acini frequently pro-

duces only localized fat necrosis or a small haemorrhage, and in our series there are several cases in which severe attacks of upper abdominal pain occurred in association with small, localized accidents of this sort, and in the absence of gall-stones, cholecystitis or fatal disease of the pancreas. These localized accidents often involve only a very limited portion of tissue and are not fatal. One not infrequently finds small organizing or organized fat necroses or haemorrhages in association with metaplasia and duct obstruction in patients who die of causes remote from the pancreas. Indeed, as we have said, there are several cases of haemorrhagic pancreatitis in our series in which, on search through a number of sections, similar old foci of encapsulated fat necrosis or haemosiderin-laden scars, resulting from acinar ruptures prior to the terminal rapidly fatal one, were found, sometimes in association with small vessels with healed areas of necrosis in their walls and containing organized thrombi. In two such cases there was a history of several severe attacks of upper abdominal pain during some months before the final fatal pancreatitis, and in neither of these cases was cholelithiasis or biliary tract disease found at autopsy. It may be remarked that it is an old clinical observation that patients who develop haemorrhagic pancreatitis have frequently a history of having had attacks of epigastric pain preceding the fatal pancreatitis. Even though the material at our disposal for study is limited, we feel strongly that it provides evidence that a severe attack of upper abdominal pain, occurring particularly in obese individuals or alcoholics, and especially following a meal or the ingestion of alcohol, may be the result of rupture of dilated acini behind an obstruction, with consequent escape of secretion and the production of a small, localized lesion of the type described above. Doubtless, many such attacks are believed, clinically, to be caused by disease of the biliary tract rather than of the pancreas.

SUMMARY

1. In human and experimental haemorrhagic pancreatitis there occurs a constant and specific vascular lesion, characterized by rapid necrosis of the walls of arteries and veins. The pancreatic haemorrhage results from the rupture of the necrotic vessels.

2. This vascular necrosis is the direct result of the action of pan-

creatic juice upon the walls of the vessels, and the agent in the juice responsible for the necrosis is the tryptic ferment.

3. A precisely similar lesion can be produced in vessels elsewhere in the body by the local injection of purified trypsin, or of pancreatic juice obtained from the duct through a cannula. The activation of trypsinogen by enterokinase is not necessary for the production of the vascular necrosis. Inactivation of trypsin by heat destroys its necrotizing power.

4. Aside from the rare instances of primary rupture or occlusion of the pancreatic blood vessels, haemorrhagic pancreatitis occurs only as a result of the disruption of the duct-acinar system with consequent escape of the secretion into the interstitial tissue of the gland.

5. The majority of cases of haemorrhagic pancreatitis result from partial obstruction to the outflow of the secretion, causing distension and rupture of acini and ductules behind the obstruction, with resulting escape of pancreatic juice into the interstitial tissue. If the escaping juice is rich in tryptic ferment and comes into contact with arteries and veins, their walls will be destroyed, and extensive or localized haemorrhage will occur depending upon the size of the affected vessels. If no arteries or veins are present in the vicinity of the acinar rupture, or if the escaping secretion has a low tryptic power, fat necrosis, produced by the lipase of the juice, may be the only result.

6. Rupture of dilated, thinned-out acini behind an obstruction is particularly liable to occur during the periods of increased pressure within the system resulting from stimuli which greatly increase the production of secretion (*e.g.*, a large meal, or the ingestion of alcohol).

7. While the underlying obstruction in some cases of haemorrhagic pancreatitis is caused by a gall-stone lodged at or near the ampulla of Vater, in most cases the main pancreatic duct is unobstructed, and the obstruction is situated in branches of the duct within the pancreas. Retrojection of bile into the pancreatic duct is an infrequent cause of haemorrhagic pancreatitis in our experience, and proved cases in the literature are scarce. Even in the cases in which bile retrojection does occur, haemorrhagic pancreatitis will not result unless the duct-acinar system is ruptured and a secretion with sufficient tryptic potency to produce vascular necrosis escapes.

8. Metaplasia of the epithelium of the branches of the pancreatic duct, leading to partial obstruction and consequent dilatation of the acini and ductules behind the obstruction, is a very frequent occurrence, having been found in our routine sections in 18.6 per cent of 150 consecutive autopsies on individuals over 25 years of age. Rupture of the distended acini behind the obstructing metaplasia also occurs frequently, but in most cases the resulting damage is limited to focal fat necroses or small haemorrhages. There is evidence that acute attacks of severe epigastric pain may be associated with these accidents.

9. Metaplasia of duct epithelium with associated acinar dilatation was found in the routine sections in 13 of 24 cases of haemorrhagic pancreatitis. It is our belief that this form of duct obstruction leading to acinar rupture and escape of secretion plays an important rôle in the pathogenesis of many cases of haemorrhagic pancreatitis, though any of the numerous causes of duct obstruction may, of course, lead to the same result.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

PLATE 1

FIG. 1. Artery in pancreas of human haemorrhagic pancreatitis (autopsy 11675, table I) showing necrosis of almost the entire circumference.

FIG. 2. Artery in pancreas of human haemorrhagic pancreatitis (autopsy S.H. 457, table I) showing necrosis of only a segment of the wall.

FIG. 3. Artery in pancreas of human haemorrhagic pancreatitis (autopsy 11675, table I) showing complete destruction and rupture of a segment of the wall with haemorrhage.

FIG. 4. Artery in pancreas of experimental haemorrhagic pancreatitis produced by injection of trypsin into pancreatic duct (dog 18), showing necrosis and hyalinization of a segment of the wall.

FIG. 5. Another artery in pancreas of same animal. Necrosis of lower side and right end.

FIG. 6. Necrosis of artery in subcutaneous tissue (dog 29 A) 24 hours after the local injection of commercial trypsin. Very few muscle fibres remain.

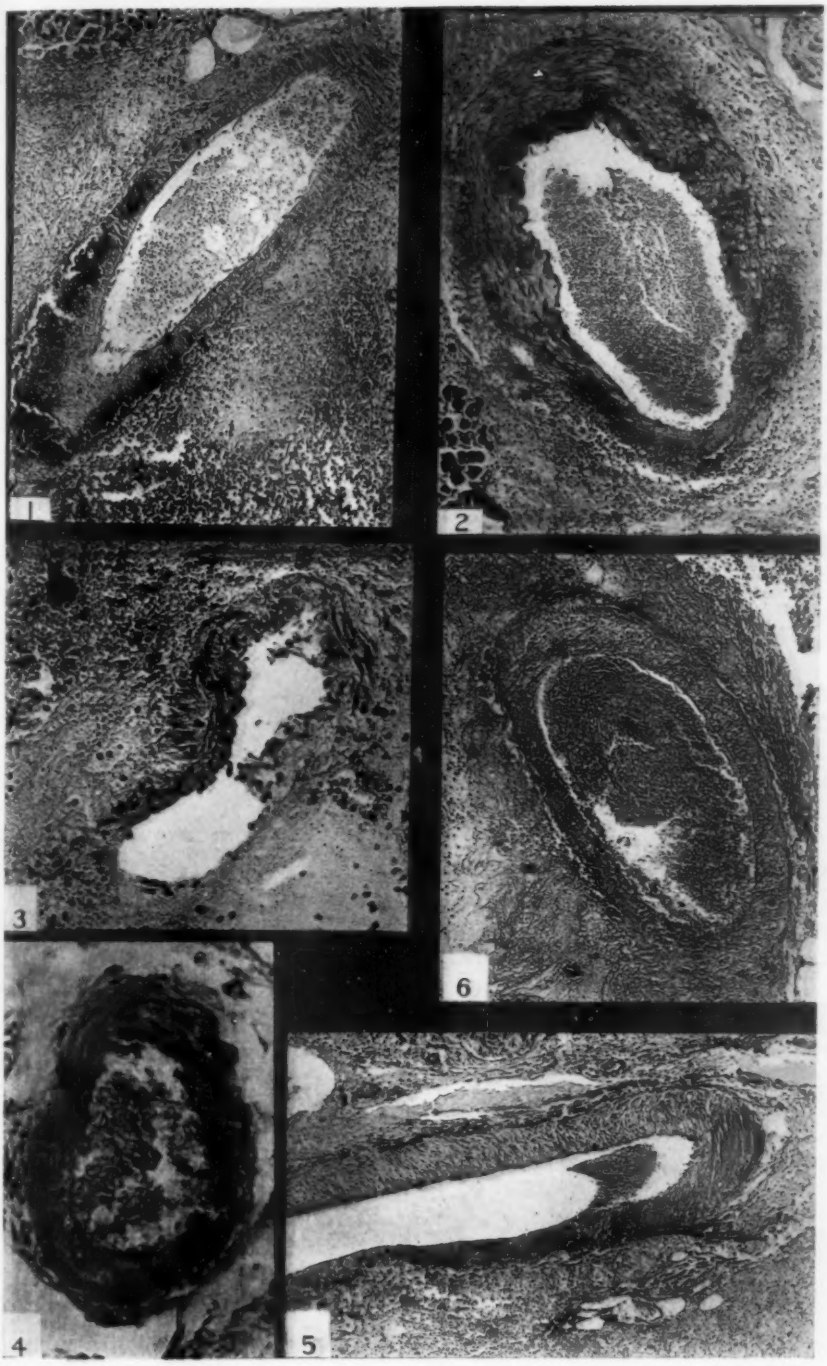
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PLATE 2

FIG. 7. Complete necrosis of artery in subcutaneous tissue (dog 38) 24 hours after the local injection of purified crystalline trypsin (Northrop). No muscle fibres remain.

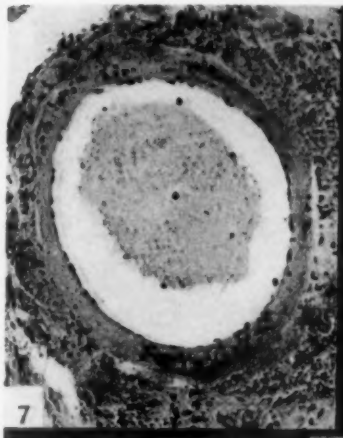
FIG. 8. Necrosis of part of wall of artery in subcutaneous tissue (dog 36) 24 hours after the local injection of sterile, unactivated pancreatic juice obtained from pancreatic duct by cannulation outside the duodenum.

FIG. 9. Necrosis of most of wall of artery in subcutaneous tissue (dog 19 P) 24 hours after the local injection of sterile, unactivated pancreatic juice obtained from pancreatic duct by cannulation outside the duodenum. The muscle fibres in a small segment of the wall on the left are fairly well preserved. Elsewhere they are almost all completely destroyed.

FIG. 10. Necrosis of wall of vessel in subcutaneous tissue of dog of figure 9, with rupture and haemorrhage. The partly necrotic portion of the wall that remains ends at the points indicated by the arrows.

FIG. 11. Metaplasia of epithelium of branches of pancreatic duct (autopsy 13167). See figure 17.

FIG. 12. Metaplasia of epithelium of pancreatic duct (autopsy 12752). At the left there is a nodule of metaplastic epithelium that grows down beneath the normal epithelium. Such down-growths are common. To the right are nodules projecting into the lumen. The apparently detached nodule is attached to the wall in further sections. The patient was a chronic alcoholic, and there are dilated acini and fat necrosis in other parts of the same section.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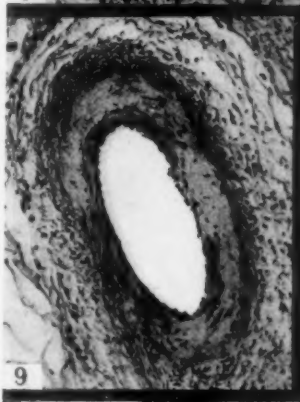
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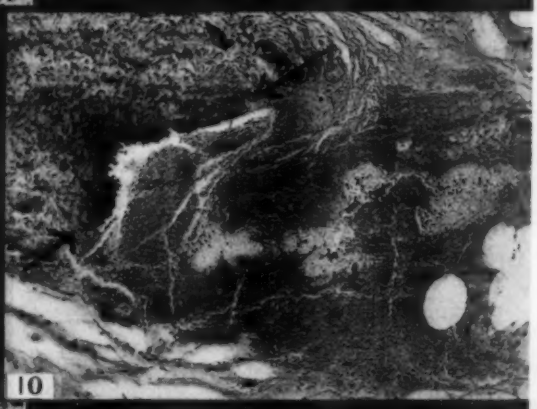
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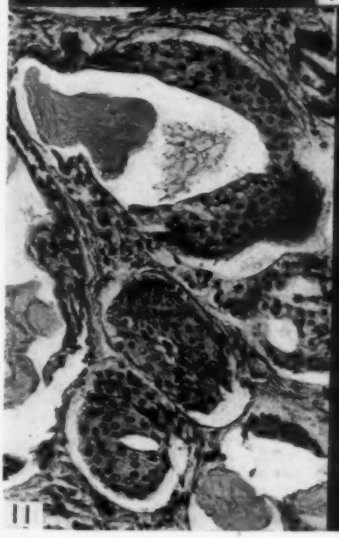
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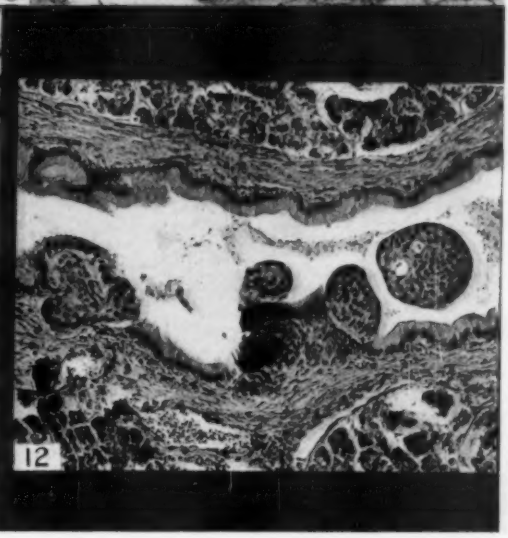
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PLATE 3

FIG. 13. Dilated ductule containing inspissated secretion in case of haemorrhagic pancreatitis (autopsy 12240, table I). To the left is a ductule with lumen greatly narrowed by concentric metaplasia.

FIG. 14. Metaplasia of duct epithelium in case of haemorrhagic pancreatitis (autopsy 13103, table I). The print is dark, and arrows have been placed to indicate the sites of metaplasia.

FIG. 15. Metaplasia of duct epithelium in case of haemorrhagic pancreatitis (autopsy 10593, table I). Many ductules were narrowed or completely occluded in this pancreas. Arrows point to metaplastic nodules. To the right there is a part of the scarred remains of a lobule, the acini of which had disappeared as a result of the occlusion of the ductules by metaplasia.

FIG. 16. Dilatation of acini behind ductule partly obstructed by metaplasia.

FIG. 17. Dilated ductules resulting from obstructing metaplasia proximal to those in the section are seen at the left. A fat necrosis resulting from rupture of dilated acini is seen at the right (arrow). This is the same case (autopsy 13167) as that of figure 11 which shows the metaplasia with a higher magnification.

FIG. 18. Duct from one of the cases of haemorrhagic pancreatitis described in the text in which the ducts contain inspissated secretion and in which liquefaction of the wall with invasion of leucocytes follows the loss of the lining epithelium. One segment of the wall of this duct has been destroyed while the rest remains intact. Masses of inspissated secretion can be seen separated by leucocytes. There are no bacteria (autopsy 10176, table I).

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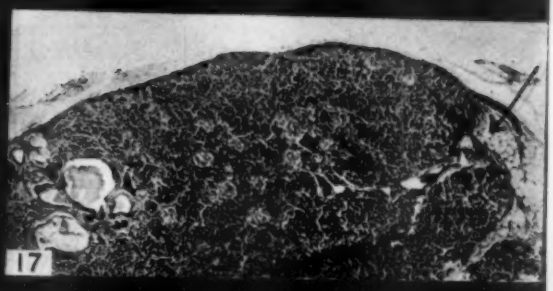
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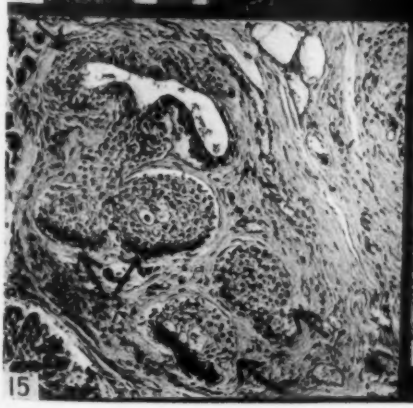
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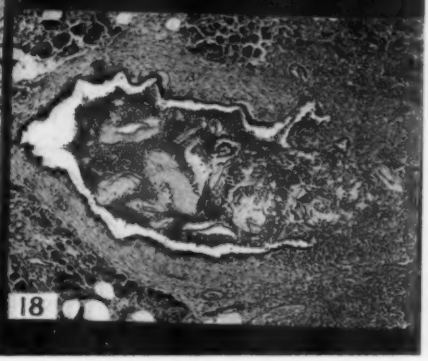
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PROGRAMS

Meeting of The Johns Hopkins Medical Association

Baltimore, Thursday, February 20—Friday, February 21, 1936

GENERAL PROGRAM

Thursday. Scientific meetings, operative clinics, etc. (*See Special Programs*)

1.00 p.m. Luncheon. Welch Medical Library. (Courtesy of the Medical School.)

5.00 p.m. University Lecture by Dr. Anson Phelps Stokes, Dean of the Washington Cathedral. (Latrobe Hall, Homewood.)

7.30 p.m. Dinner of the Medical and Surgical Associations. The Alcazar.
Toastmaster—Dr. Alan M. Chesney, Dean of the Medical School.
Speakers—Dr. Isaiah Bowman, President of the University.
Dr. C. Sidney Burwell, Professor of Research Medicine and Dean of the Harvard Medical School.
Dr. Thomas M. Rivers, Member of the Rockefeller Institute.

Friday. Scientific meetings, operative clinics, etc. (*See Special Programs*)

1.00 p.m. Luncheon Auditorium, 5th floor, Phipps Clinic. (Courtesy of the Hospital.)

5.00 p.m. University Lecture by Dr. Anson Phelps Stokes. (Latrobe Hall, Homewood.)

8.30 p.m. Address at the Lyric by Dr. George E. Vincent, Former President of the Rockefeller Foundation.

SPECIAL PROGRAM—MEDICAL SECTION

Thursday Morning—Hurd Memorial Auditorium

9.30 Presentation of Cases. Warfield T. Longcope, Professor of Medicine. E. P. Carter, G. A. Harrop, W. S. Tillett and House Staff.
X-Ray Demonstration. Dr. J. W. Pierson.

10.30 Modes of Development of Macrocytic Anemia. M. M. Wintrobe.

11.00 Virus Uveitis. Alan C. Woods, Acting Professor of Ophthalmology.

11.20 Lymphogranuloma Inguinale. Paul Padget.

11.40 Syphilitic Nephritis. Benjamin M. Baker and Warde B. Allan.

12.00 Haemoglobinaemia. Alan Bernstein.

12.15 Present Trends in Obstetric Analgesia. Nicholas J. Eastman, Professor of Obstetrics.

12.30 Business meeting.

1.00 Luncheon. Welch Medical Library.

Thursday Afternoon—Lecture Room, 2nd floor, Pathology Bldg.

2.30 Symposium on Female Sex Hormones.
The General Relationship of the Anterior Pituitary and Ovary to Reproduction. PHILIP BARD, Professor of Physiology.
Sex Hormones and Menstruation. CARL G. HARTMAN.
Oestrogenic Hormones in the Treatment of Gonorrheal Vaginitis in Children. RICHARD W. TE LINDE.
The Prolongation of Gestation in the Rabbit by Human Pregnancy Urine. FRANKLIN F. SNYDER.

3.30 Cellular Oxidation-Reduction and Control of Hydrolytic Processes and Lysis. W. MANSFIELD CLARK, Professor of Physiol. Chem. LESLIE HILLERMAN and BARNETT COHEN.

5.00 Lecture. Origin of Universities and Their Development in the Middle Ages and the Renaissance. DR. ANSON PHELPS STOKES. (Latrobe Hall.)

7.30 Dinner. The Alcazar.

Friday Morning—Hurd Memorial Auditorium

10.00 Some observations on the Effect of Circulation on Skin Reactions. LESLIE N. GAY and FRANK B. FURSTENBERG.

10.15 The Incidence of Throat Infections in Various Diseases. JOHN T. KING.

10.30 Clinical Types of Minute Beta Hemolytic Streptococcus Infection. PERRIN H. LONG AND ELEANOR A. BLISS.

11.00 Adrenalectomized Rats. CURT P. RICHTER.
Discussion. GEORGE A. HARROP.

11.30 Progressive Exophthalmos Following Subtotal Thyroidectomy. HENRY M. THOMAS, JR. and ALAN C. WOODS.

11.45 Acute Pulmonary Fibrosis. LOUIS HAMMAN and ARNOLD R. RICH.

12.00 The Intravenous Administration of Fat. L. EMMETT HOLT.

12.15 The Medical Practitioner's Opportunity and Responsibility in the Matter of Admission of Patients to Psychiatric Hospitals. WENDELL S. MUNCIE.

1.00 Luncheon. Auditorium, 5th floor, Phipps Clinic.

Friday Afternoon

2.30 Laboratory Demonstrations. At the request of five or more members, made to Dr. H. M. THOMAS, JR. before 2.30 p.m. Thursday, demonstrations will be arranged in any of the following laboratories:

a. Cardiological Laboratory—DR. EDWARD P. CARTER.
b. Clinical Chemical Laboratory—DR. GEO. A. HARROP.
c. Clinical Bacteriological Laboratory—DRS. W. S. TILLETT and P. H. LONG.
d. Haematological Laboratory—DR. M. M. WINTROBE.

5.00 Lecture. The Development of Universities in Europe and America from the Protestant Reformation to the Present. Dr. Anson Phelps Stokes. (Latrobe Hall.)

8.30 Lecture. The Few and the Many in Education. Dr. George E. Vincent. (The Lyric.)

SPECIAL PROGRAM—PEDIATRIC SECTION—HARRIET LANE AMPHITHEATER

Thursday, February 20, 9 a.m.

1. Borotartrate in the Treatment of Epilepsy. Laslo Kajdi.
2. Observations on Blood Iron. Hugh W. Josephs.
3. The Hypothyroid Factor in Growth and Osseous Development. Lawson Wilkins.
4. Criteria for Discontinuing Antimeningococcic Therapy. Francis F. Schwentker.
5. Arteriolar Sclerosis in Childhood. Harriet G. Guild.
6. Observations on Hypertension in Children. Helen B. Taussig.
7. A Study in Dehydration. Horace L. Hodes.
8. Interpretation of Glucose Tolerance Curves. Edward M. Bridge.
9. Renal Rickets and Hyperparathyroidism. Alexander J. Schaffer.
10. Mortality in Tuberculin Positive Infants. Miriam E. Brailey.
11. Essential Lipemia. Francis X. Aylward and L. Emmett Holt.

MEETING OF THE JOHNS HOPKINS SURGICAL ASSOCIATION

Baltimore, Thursday, February 20—Friday, February 21, 1936

GENERAL PROGRAM

Thursday, February 20

9.00 a.m. Operative Clinics. See Special Program.

1.00–2.00 p.m. Luncheon. (William H. Welch Memorial Library.) Courtesy of Johns Hopkins Medical School.

2.00–4.00 p.m. Scientific Papers. (Hurd Memorial Auditorium.)

7.30 p.m. Biennial Dinner. (The Alcazar.)

Friday, February 21

9.00 a.m.–12.00 noon. Operative Clinics. See Special Program.

12.00 noon. Surgical Clinic. Dr. Lewis.

1 p.m. Luncheon. (Auditorium, 5th floor Phipps Clinic.) Courtesy of Johns Hopkins Hospital.

2.00 p.m. Scientific Papers. (Hurd Memorial Auditorium.)

SPECIAL PROGRAM

Thursday

9.00 a.m. Operative Clinics: Drs. Lewis, Cullen, Dandy, Fisher, Hunner, Rienhoff, Te Linde, Young.
Scientific Papers. (Hurd Memorial Auditorium)
2.00 p.m. Dr. G. E. Bennett. Old Unreduced Dislocation of the Shoulder.
2.15 Dr. W. F. Rienhoff, Jr. Surgical Technique in Total and Subtotal Pneumonectomy.
2.30 Dr. C. F. Geschickter. Hormones in Relation to Tumors.
2.45 Dr. F. C. Lee. Nerve Compression.
3.00 Dr. H. H. Young. A New Surgical Approach to the Adrenals. Simultaneous Exposure of Both Adrenals. Presentation of Cases with Bilateral and Unilateral Resection (Slides).
3.15 Dr. I. R. Trimble. Gastro-Enterostomy in the Treatment of Gastric and Duodenal Ulcers.
3.30 Drs. A. Hebb and S. McLanahan. Ano-Rectal Strictures due to Lymphopathia Venerea (Lymphogranuloma Inguinale).
3.45 Dr. B. Woodhall. Intracranial Hypertension Associated with Lateral Sinus Thrombosis.
4.00 Dr. P. A. Kunkel, Jr. Chronic Subdural Hematoma.
7.30 Dinner of the Surgical and Medical Associations. (The Alcazar, Madison and Cathedral Streets.)

Friday

9.00 a.m. Operative Clinics: Drs. Finney, Bennett, Casler, Crowe, Davis, Richardson, Stone.
12.00 Noon Surgical Clinic. Dr. Lewis. (Surgical Lecture Room.)
1.00 p.m. Luncheon. (Auditorium, 5th floor Phipps Clinic.)
Scientific Papers. (Hurd Memorial Auditorium)
2.00 Dr. A. C. Woods. Demonstration of Operations by Colored Motion Pictures.
2.15 Dr. P. Bard. Neurological Mechanism Underlying Oestrual Behavior (Motion Picture).
2.40 Drs. E. K. Marshall and M. Rosenfeld. Depression of Respiration by Oxygen.
3.00 Dr. A. R. Rich. Studies on the Pathogenesis of Hemorrhagic Pancreatitis.
3.20 Dr. A. B. Howell. The Phylogeny of the Forearm Musculature (Slides).
3.40 Drs. A. Grollman and W. M. Firor. The Adrenal Cortical Hormone.

JOHNS HOPKINS UNIVERSITY PROGRAM

February 20

5.00 p.m. THE REVEREND ANSON PHELPS STOKES, Canon of Washington Cathedral. The Origin of Universities and Their Development in the Middle Ages and the Renaissance. (Latrobe Hall, Homewood.)

February 21

5.00 p.m. CANON STOKES. The Development of Universities in Europe and America from the Protestant Reformation to the Present. (Latrobe Hall, Homewood.)

8.30 DR. GEORGE E. VINCENT, Former President of the Rockefeller Foundation. The Few and the Many in Education. (The Lyric, Mount Royal Avenue.)

February 22

11.00 a.m. Commemoration Exercises and Conferring of Honorary Degrees. Speaker: ISAIAH BOWMAN, President of the Johns Hopkins University. (The Lyric.)

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

A Marriage Manual. By Drs. Hannah and Abraham Stone. 334 pp. $2.50. (*Simon & Schuster, New York, 1935.*)

It is a rare privilege for a reviewer to find himself in the position to praise a practical book on matters of sex; most authors merely disguise pornographic content with a lofty scientific title. It is not so with "A Marriage Manual," by Dr. and Mrs. Stone, who do a solemn job in a very conscientious manner. The authors are well qualified to write such a book since they have had clinical experience with the material contained, both of them having been associated for years with several of the Marriage Consultation Centers in New York City.

The book begins with a discussion of the individual's fitness for marriage, stressing hereditary factors. This is followed by a chapter on the biology of human reproduction. They conclude the book with chapters on contraception and the adjustment of sexual disharmonies. This last topic is treated fully and, as far as I know, the authors have given us the best exposition of it for the layman. Both Dr. and Mrs. Stone have an unusually intelligent and tolerant point of view and it is well worthwhile for medically trained readers to read carefully these particular pages; the book is a safe and helpful manual for us to recommend to our patients.

The reviewer finds one minor objection; the book is styled as a catechism using the question and answer method. This sacrifices smoothness and literary tone to lucidity, all of which could be better obtained by skillful writing on the unbroken page.

A. F. G.

Diseases of the Thyroid Gland. By Arthur E. Hertzler. Edited by Victor E. Chesky. 3rd Ed. 348 pp., illus. $7.50. (*C. V. Mosby Co., St. Louis, 1935.*)

This is a highly practical discussion of the surgical treatment of the diseases of the thyroid which are grouped according to a classification devised by the author. Admitting that nothing is known of the cause of goitre, the subdivisions are based largely on the symptoms produced, and the terms toxic or non-toxic are used to designate the types. The studies were made in a community where the patients are conservative residents for many years so that the course of the disease and the results of treatment could both be followed. The chapter on surgical technique is carefully worked out, and, on the whole, until further studies reveal the causes of these affections, the forms of treatment suggested must be regarded as the best available.

M.